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Structure and Functional Potential of Microbial Communities in Subglacial Lake Whillans and at the Ross Ice Shelf Grounding Zone, West Antarctica

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STRUCTURE AND FUNCTIONAL POTENTIAL OF MICROBIAL COMMUNITIES IN SUBGLACIAL LAKE WHILLANS AND AT THE ROSS ICE SHELF GROUNDING ZONE, WEST ANTARCTICA

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ABSTRACT

The abundance of liquid water found beneath the Antarctic Ice Sheet (AIS) is thought to provide a suitable habitat for microbial life. However, the thick ice sheet has posed a significant obstacle to accessing this isolated system and as a consequence, little data exists to infer ecosystem processes occurring beneath ice sheets. To address this deficit, data generated through 16S rRNA (genes and molecules) and metagenomic sequencing was used to evaluate the diversity and potential metabolic function of communities inhabiting Subglacial Lake Whillans (SLW) and the ocean basin into which it discharges (Whillans Ice Stream grounding zone; WIS-GZ). The analysis of SLW was found to be dominated by bacterial and archaeal species with little evidence for active eukaryotic organisms. The abundance of taxa related to chemolithoautrophic species (e.g., *Thiobacillus*, *Sideroxydans, Nitrosospira*, and *Candidatus* Nitrosoarcheaum) suggested that reduced sulfur, iron, and nitrogen compounds were important in fueling primary production in the permanently dark lake. Consistent with this, genes involved in inorganic carbon fixation, sulfide oxidation, and ammonia oxidation were also identified and taxonomically affiliated with species of *Thiobacillus* and *Nitrosospira*. Furthermore, the abundance of sequences related to *Methylobacter* combined with the detection of methanogenic taxa provided evidence for methane cycling beneath the AIS. Although organic carbon and nutrients generated in SLW are transported to the WIS-GZ and may help support the diverse community found there, the prevalence of chemolithoautotrophic taxa (e.g., *Thioprofundum* and *Thiohalophilus*) suggests primary production coupled to the oxidation of reduced sulfur compounds may also provide a source of new fixed carbon to the system. The WIS-GZ community was distinct from those of SLW and the Ross Ice Shelf edge, signifying that it is structured based on the combined influences of input from the subglacial and marine systems. Collectively, these studies show that diverse microbial assemblages exist beneath ice sheets that are largely sustained through chemosynthesis and the weathering of bedrock minerals.

CHAPTER 1.
INTRODUCTION

Distribution and Characteristics of Subglacial Aquatic Environments

Currently, 11% of the planet is covered by polar ice sheets and mountain glaciers. The environments beneath such ice masses have been found to contain an abundance of liquid water (Iken and Bindschadler, 1986; Wright and Siegert, 2012; Chu, 2014) that saturates sediments, accumulates in topographical lows forming lacustrine features, and creates drainage conduits along hydropotential gradients (Fricker et al, 2007; Wright and Siegert, 2012; Carter et al, 2013). The presence of liquid water beneath the ice sheets of Greenland and Antarctica has implications not only for the stability of the ice masses (Bindschadler, 1983), but also the habitability of subglacial environments by microorganisms (Priscu et al, 1999; Sharp et al, 1999).

The Greenland Ice Sheet (GRIS) subglacial hydrologic system, and that of many temperate glaciers, is thought to be predominantly characterized by diffuse and channelized drainage networks that are supplied by surficial melt (Chu, 2014). Over the course of the melt season, liquid water from the surface of the ice sheet may drain to the base through ice crevasses and moulins (Chu, 2014). Although the behavior of water at the bed is poorly characterized, it is thought that drainage networks evolve over time from the slow, inefficient transfer of water between connected cavities to that of an efficient channelized flow as meltwater erodes the glacial ice and underlying sediments (Chandler et al, 2013; Chu, 2014). Water eventually exits the subglacial environment to form proglacial streams and lakes, or for marine-terminating glaciers, flows directly to the ocean (Chu, 2014).

The subglacial hydrologic network beneath the Antarctic ice sheet (AIS) is distinct from that observed in Greenland and other glaciers, with one notable differences being the subglacial water source. In Antarctica, water at the bed is derived from basal melting, as there is no significant input of melt water from the surface. Widespread melting occurs beneath the AIS (Llubes et al, 2006; Bell et al,

2008) where the base of the ice sheet is at the pressure melting point and in regions of adequate geothermal heat flux (Siegert, 2001; Fisher et al, 2015). This abundance of liquid water is partially housed in the extensive network of subglacial lakes and wetlands that exist under the AIS (Wright and Siegert, 2012).

Using radio echo sounding, a technique that enables measurement of the ice thickness and identification of layers of liquid water (Robin et al, 1970; Oswald and Robin, 1973), subglacial lakes have been found to be widely distributed beneath the AIS and are largely concentrated along ice divides or associated with fast moving glaciers (i.e., ice streams; Wright and Siegert, 2012). The lakes range in size from < 1km to ~240km long, the largest being Subglacial Lake Vostok (SLV) beneath the East Antarctic Ice Sheet (EAIS; Siegert et al, 2001). In Greenland, only a handful of small subglacial lakes have been identified, the first of which was not reported until 2013 (Palmer et al, 2013; Palmer et al, 2015).

Water residency times within some Antarctic lakes are estimated to be as much as tens of thousands of years (Siegert et al, 2004; Thoma et al, 2008) while others, including those found in Greenland, are more dynamic and experience high turnover rates (i.e., years), making them 'active' lakes (Fricker et al, 2007; Smith et al, 2009, Palmer et al, 2015). Water flux into and out of such lakes during episodic fill and drain cycles can be detected as surface elevation changes in the overlying ice sheet, measured by satellite laser altimetry (e.g., Fricker et al. 2007). Using this method, approximately 130 active subglacial lakes have been identified and predominantly found at the coastal margins of the AIS where subglacial water eventually drains into the surrounding ocean (Smith et al, 2009). The storage and sudden drainage of subglacial water is known to affect ice sheet dynamics in both Antarctica and Greenland (Bell et al, 2008; Stearns et al, 2008; Sundal et al, 2011; Bartholomew et al, 2012) and water residency time is important for enabling biogeochemical reactions to occur at the bed (Tranter et al, 2002; Wadham et al, 2010).

Subglacial Environments as Microbial Habitats

Hidden beneath hundreds of meters of ice, subglacial environments are among the least accessible locations on earth. The lack of sunlight, low temperature, and limited nutrient input from the surface suggest they may be one of the most extreme habitats known. Despite these challenges, microorganisms have been detected in subglacial water and sediments (e.g., Priscu et al, 1999; Skidmore et al, 2005; Cheng and Foght, 2007; Mikucki and Priscu, 2007; Lanoil et al, 2009; Boyd et al, 2011; Christner et al, 2012; Hamilton et al, 2013; Dieser et al, 2014) from a handful of polar and mountain systems. The available data from the Antarctica subglacial environment suggest it may harbor ~10^{29} microbial cells (Priscu et al, 2008), which is similar to estimates for open ocean waters (Whitman et al, 1998). Although limited information is available to infer processes occurring at the bed of larger ice masses, microbial communities and biogeochemical cycling are hypothesized to occur beneath the world's ice sheets (Table 1.1; e.g., Skidmore, 2011; Wadham et al. 2012).

There is evidence that microorganisms in subglacial environments derive their nutrients and energy from gasses melted out of the overlying ice and the weathering of bedrock minerals liberated by glacial comminution (Skidmore 2011; Mitchell et al, 2013). In Greenland and other Arctic and mountain glacier systems, the subglacial environment may also receive input from the surface in the form of atmospheric gasses, and organic matter carried in melt waters (Stibal et al, 2008; Irvine-Flynn et al, 2012; Bhatia et al, 2013). Furthermore, comparative studies have found common taxa within supra- and subglacial microbial communities, indicating that surface populations are transported to the glacial bed (Hamilton et al, 2013; Dieser et al, 2014). The combination of autochthonous and allochthonous nutrients available appear sufficient to support a variety of microbial metabolisms in subglacial environments (Table 1.1).

Table 1.1. Reports documenting evidence of microbial metabolic activity in subglacial systems.

Metabolisms	Organisms	Analysis Methods	References
Carbon fixation	*Sideroxydans*	Functional gene, Geochemistry, Radiolabel incorporation	Gaidos et al 2004; Christner et al 2012; Boyd et al 2014
Aerobic respiration	*Polaromonas, Rhodoferax, Flavobacterium, Brevundimonas, Microbacterium*	Enrichment, Geochemistry, Radiolabel incorporation	Karl et al 1999; Skidmore et al 2000; Tranter et al 2002; Foght et al 2004; Mikucki et al 2004; Cheng and Foght 2007; Mikucki and Priscu 2007; Mitchell and Brown 2008; Lanoil et al 2009
Nitrogen fixation		Enrichment, Functional gene	Foght et al 2004; Boyd et al 2011
Nitrification	*Nitrosopumilus, Nitrosomonadales*	Functional gene, Geochemistry	Wynn et al 2007; Boyd et al 2011
Nitrate reduction	*Polaromonas*	Enrichment, Functional gene, Geochemistry	Tranter et al 1994; Tranter et al 1997; Skidmore et al 2000; Foght et al 2004; Boyd et al 2011; Montross et al 2013
Sulfur/iron oxidation	*Thiobacillus, Flavobacterium*	Enrichment, Geochemistry	Tranter et al 1997; Bottrell and Tranter 2002; Tranter et al 2002; Skidmore et al 2005; Mitchell et al 2013; Boyd et al 2014; Harrold et al 2015
Iron reduction		Enrichment, Geochemistry	Foght et al 2004; Mikucki and Priscu 2007; Mikucki et al 2009
Sulfate reduction	*Desulfocapsa*	Enrichment, Functional gene, Geochemistry	Skidmore et al 2000; Wadham et al 2004; Mikucki et al 2009
Methane oxidation	*Methylobacter, Methylovulum*	Functional gene, Geochemistry	Dieser et al 2014
Methanogenesis	*Methanosarcinales*	Enrichment, Functional gene, Geochemistry	Skidmore et al 2000; Boyd et al 2010; Christner et al 2012; Stibal et al 2012; Dieser et al 2014

Arctic and Alpine Subglacial Environments

The microbial communities documented in water and sediment from beneath Arctic and Alpine glaciers are bacterially dominated and largely consist of *Proteobacteria* (i.e., *Beta-*, *Gamma-*, and *Alpha-*

), *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* (Foght et al, 2004; Skidmore et al, 2005; Cheng and Foght 2007; Stibal et al, 2012a; Hamilton et al, 2013; Dieser et al, 2014). Archaeal and eukaryotic species have only been examined in a few studies (Stibal et al, 2012a; Hamilton et al, 2013; Dieser et al, 2014), and although they may have crucial biogeochemical roles (e.g., Dieser et al, 2014), they are thought to be minor components of these communities. Aerobic respiration and heterotrophic metabolism by members of subglacial microbial assemblages (Table 1.1) have been demonstrated in enrichment cultures (Skidmore et al, 2000; Foght et al, 2004; Skidmore et al, 2005; Cheng and Foght, 2007) as well as radiolabeled nutrient incorporation experiments (Skidmore et al, 2000; Foght et al, 2004) and have been inferred from geochemical measurements (Tranter et al, 2002; Mitchell and Brown, 2008). The organic carbon needed to sustain these populations may be found in sediment deposits (e.g., Stibal et al, 2012b), be transported in supraglacial melt (e.g., Stibal et al, 2008), or produced *in situ* by autotrophic activity (Boyd et al, 2014).

Due to the lack of sunlight, primary production in the subglacial environment is restricted to chemosynthesis. Based on geochemical (Tranter et al, 2005; Skidmore et al, 2010; Mitchell et al, 2013) and molecular data (Table 1.1; Skidmore et al, 2005; Boyd et al, 2014; Harrold et al, 2015), the oxidation of iron and sulfur minerals (e.g., pyrite) by microorganisms may be a common process occurring beneath glaciers that further enhances the weathering of bedrock minerals (Skidmore, 2011). Evidence suggests that sulfide oxidation occurs aerobically or anaerobically via the reduction of ferric iron or nitrate (Tranter et al, 1994; Tranter et al, 1997; Bottrell and Tranter, 2002; Boyd et al, 2011 Montross et al, 2013). Although nitrate may originate from snowmelt (Tranter et al, 1994), it may also be sourced from microbial nitrification (Wynn et al, 2007). In support of this, bacterial and archaeal ammonia oxidation activity have been documented in laboratory incubations of subglacial sediments (Boyd et al, 2011; Hamilton et al, 2013).

Ice sheets have been proposed to be a globally significant source of methane, produced from the activity of methanogenic archaea in the subglacial environment (Wadham et al, 2008). A methane reservoir of ~10 Pg C may exist beneath the WAIS alone, the release of which into the atmosphere could have significant climatic implications (Wadham et al, 2012). These predictions are supported by the presence of elevated methane concentrations in glacial sediments and subglacial water outflows combined with the detection of methanogenic species (e.g., *Methanomicrobiales* and *Methanosarcinales*; Table 1.1; Boyd et al, 2010; Stibal et al, 2012b; Dieser et al, 2014). Further, evidence for the bacterial oxidation of methane in subglacial outflows suggests methane cycling is occurring beneath the GRIS (Dieser et al, 2014) and perhaps the AIS.

Subglacial Caldera Lakes

Subglacial environments within volcanic craters in Iceland are distinctly different that those studied in Greenland and Antarctica in that they receive significant active hydrothermal input. These subglacial lakes are formed beneath ice caps due to geothermal heat flux. Three lakes beneath the Vatnajökull ice cap within the Grímsvötn and Skaftárkatlar calderas have been biogeochemically characterized (Gaidos et al, 2004; Gaidos et al, 2009; Marteinsson et al, 2013). Grímsvötn was the first of the lakes to be explored and was found to contain aerobic waters, attributed to oxygen release from glacial melt (Gaidos et al, 2004). Studies detected a viable microbial community with many members related to known psychrophilic and thermophilic species and capable of chemoautotrophy (Gaidos et al, 2004). The Skaftárkatlar caldera lakes had oxygenated surface waters but the majority of the water column was sulfidic (Gaidos et al, 2009; Marteinsson et al, 2013). Both lakes were bacterially dominated with no archaea detected (Gaidos et al, 2009; Marteinsson et al, 2013). Many of the abundant organisms within the lakes were related to autotrophic species that use hydrogen or sulfur species as electron donors (e.g., *Acetobacterium*, *Sulfurospirillium*, *Sulfuricurvum*, and *Desulfosporosinus*; Gaidos et al, 2009; Marteinsson et al, 2013).

Antarctic Subglacial Aquatic Environments

The subglacial aquatic environment beneath the AIS is the largest in extent, and yet the least explored. Prior to 2013, investigations of this region were limited to three sites: Taylor Glacier at Blood Falls (Mikucki et al, 2004; Mikucki and Priscu, 2007; Mikucki et al, 2009), the Kamb Ice Stream (KIS; Lanoil et al, 2009), and Subglacial Lake Vostok (e.g., Priscu et al, 1999; Christner et al, 2006; Bulat, 2016; Figure 1.1). Investigations of samples collected from these sites provided the first evidence that the vast Antarctic subglacial environment is a microbial biome.

Blood Falls is an outflow feature of the Taylor Glacier in the McMurdo Dry Valleys and was named because the iron rich subglacial outflow emanating from the glacier terminus turns an orange/red color when oxidized by atmospheric oxygen (Mikucki et al, 2004; Figure 1.1). The source of the hypersaline water is thought to be an anoxic brine derived from the concentration of marine waters that inundated this region during the Pliocene (Mikcuki et al, 2004; Mikucki et al, 2015). Microorganisms within the outflows were active and capable of heterotrophic and autotrophic metabolisms (Mikucki et al, 2004; Mikucki and Priscu, 2007). Like many other subglacial environments, the microbial assemblages characterized at Blood Falls are largely composed of *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes,* with most of the taxa phylogenetically related to marine species, consistent with the regions geological history (Mikucki and Priscu, 2007). Several of the dominant organisms were related to taxa capable of deriving energy from iron and sulfur compounds (e.g., *Thiomicrospira* and *Desulfocapsa*); metabolisms that would be well suited for the environment where sulfur and iron cycling are thought to be tightly coupled (Mickucki and Priscu, 2007; Mikucki et al, 2009). Although studies on Blood Falls have provided valuable insight into a unique microbial habitat that has been likened to an ancient ferrous ocean (Mikucki et al, 2009), it is not thought to be representative of the larger Antarctic subglacial environment.

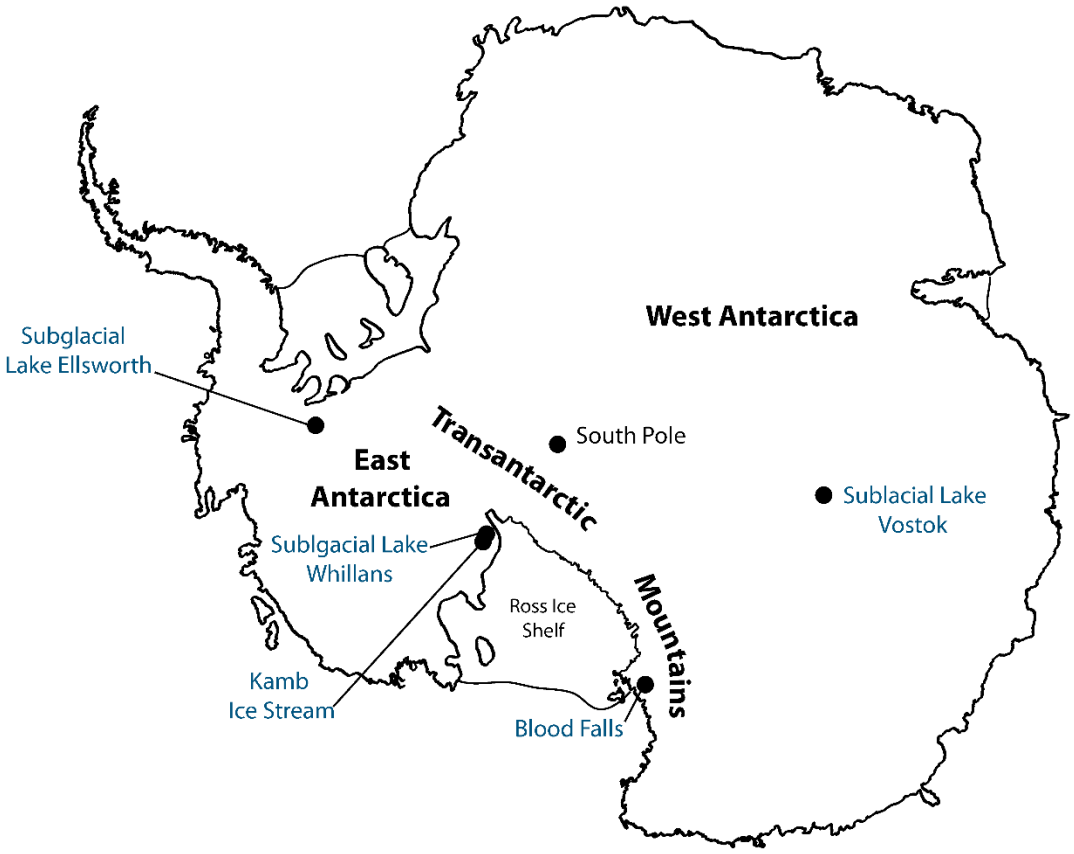


Figure 1.1 Locations of Antarctic subglacial sites discussed (blue).

The KIS, located on the Siple Coast of West Antarctica is one of several ice streams that drains the WAIS and supplies ice to the Ross Ice Shelf (Figure 1.1). In early 2000, a hot water drill was used to allow access to the bed of the ice stream and the recovery of a 43cm long sediment core (Lanoil et al, 2009). Although the sediment core was initially collected with the aim of understanding how the physical conditions at the bed affected the movement of the overlying ice stream, its recovery provided a rare opportunity to also explore the microbial assemblages beneath ice sheets (Lanoil et al, 2009). The water saturated sediment core was found to contain an abundant (~10^7 cells g^{-1}) community composed of *Betaproteobacteria*, *Alphaproteobacteria*, and *Actinobacteria* (Lanoil et la, 2009). Given that the sample was not originally intended for use in a microbiological analysis, the anomalously high cell concentration and low diversity was attributed to prolonged storage of the core prior to analysis (i.e.,

4˚C for 15 months). Despite this caveat, the internal portion of the core that was analyzed was not thought to be introduced to biological contamination from drilling or handling therefore, the organisms identified and cultured from the sample were considered to represent native species (Lanoil et al, 2009). Viable heterotrophic organisms were recovered, the majority of which were most closely related to taxa previously identified in subglacial environments (e.g., *Afipia* and *Polaromonas*; Lanoil et al, 2009). Additionally, molecular analysis revealed the presence of *Gallionella* and *Thiobacillus* species that were related to cultured representatives capable of iron and sulfide oxidation (Lanoil et al, 2009). Based on these results, it was concluded that chemolithotrophic activity combined with the mineralization of sedimentary organic carbon could support microbial communities beneath the ice (Lanoil et la, 2009).

Most of what is known about Antarctic subglacial aquatic environments is based on data from Subglacial Lake Vostok (SLV), which is located in East Antarctica beneath nearly 4km of ice (Figure 1.1). SLV is the largest of all Antarctic subglacial lakes and also one of the largest freshwater lakes on Earth, with an estimated volume of 5400 ± 1600km^3 (240km long and 50km wide; Siegert et al, 2001; Studinger et al, 2004). The lake consists of a shallow northern basin (~500m deep) and a larger southern basin (~800m deep) that are separated by a submerged ridge (Studinger et al, 2004). The variability in the thickness of the overlying ice sheet results in basal melting over the north basin and freezing of lake water (i.e., accretion) over the south basin (Siegert et al, 2000; Studinger et al, 2004). The melting of the overlying ice sheet is thought to be an important source of gasses and nutrients for the community (Siegert et al, 2001). Indeed, speculation has suggested that over time, the lake may accumulate enough gasses to generate clathrates and become supersaturated with regards to atmospheric oxygen, posing a significant physiological stress to the lake inhabitants (McKay et al, 2003). In addition to input from glacial melt, it has been proposed that geothermal fluids may also enter the lake through faults, providing chemical energy sources important to sustaining an active community (Bulat et al, 2004).

Most of what is known about the biological and limnological characteristics of SLV has been inferred from analysis of ~200m of accretion ice that was formed from lake water freezing to the base of the ice sheet (Jouzel et al, 1999; Karl et al, 1999; Priscu et al, 1999; Siegert et al, 2001; Christner et al, 2001; Bulat et al, 2004; Christner et al, 2006). This accretion ice was fortuitously discovered during the coring of the EAIS for climate records and provided a means to examine the system prior to direct exploration. Core samples of the accretion ice contained between 10^2-10^4 cells mL^{-1} (Karl et al, 1999; Priscu et al, 1999) and radiolabel nutrient incorporation assays indicated the presence of metabolically active microorganisms (Karl et al, 1999; Christner et al, 2006). Low concentrations of organic carbon were present in the accretion ice, suggesting that there was insufficient carbon entering the system to sustain an active heterotrophic population (Karl et al, 1999; Priscu et al, 1999; Christner et al, 2006). Molecular analysis revealed that the ice was dominated by bacteria (*Proteobacteria, Firmicutes*, *Actinobacteria*, and *Bacteroides*) with few archaeal or eukaryotic members (Priscu et al, 1999; Christner et al, 2001, 2006; Rogers et al, 2013). However, given uncertainties regarding the partitioning of cells, particulates, and chemical compounds during the accretion process, it is unclear how representative these samples are of the *in situ* SLV water column. Furthermore, the cores were collected using an electrochemical drill with a kerosene drill fluid which inevitably contaminated the samples, hindering analysis (e.g., Alekhina et al, 2007).

In 2012, drilling progressed through the accretion ice and into SLV (Bulat, 2016). Upon entry, lake water entered the borehole, froze, and a portion of this material was retrieved the following year by ice coring. Although the recovered samples were found to be contaminated with the kerosene drilling fluid (Bulat, 2016), a conservative microbiology analysis was conducted on the refrozen water and suggested the lake likely contained tens of cells mL^{-1} (Bulat, 2016). In 2015, that lake was drilled into again and refrozen lake was recovered from the borehole (Bulat, 2016). Analysis of these samples has yet to be reported; however, due to the manner in which the lake was accessed and sampled, only

limited information can be gained. The biogeochemical characteristics of the 500-800m water column and underlying sediments still remain unexplored.

Exploration of Antarctic Subglacial Lakes

Information on the microbial diversity and ecological processes occurring beneath large ice masses is based primarily on data collected at the margin (i.e., outflows). It remains to be determined if such samples are representative of the internal regions of ice masses, particularly that of the vast AIS. Thus far, samples available from beneath the AIS have been suboptimal for establishing the suitability of the environment as an ecosystem. Furthermore, with the exception of Icelandic cauldera lakes, no direct observations exists of the microbial communities conducting biogeochemical cycling in subglacial lakes. Antarctic subglacial lakes are unique in that they may persist in isolation from modern surface input for hundreds of thousands of years. An understanding of the evolution, abundance, diversity, and activity of microbial ecosystems in these environments requires direct access to make observations and retrieve pristine water and sediment samples for physical, geochemical, and microbiological analysis.

Exploration of Antarctic subglacial lakes requires surmounting a number of logistical challenges associated with lake access and sample recovery. To identify and develop strategies to overcome these challenges, international working groups were established in the late 90's (Priscu et al, 2003). From these discussions, it became clear that careful site selection was crucial to maximizing scientific value and that lake exploration must be conducted in an environmentally responsible way to ensure protection of the subglacial environment (Priscu et al, 2003; National Research Council, 2007). To inform site selection, it was necessary to have a better understanding of the geological, glaciological, and hydrological context in which lakes exist. With the expansion of remotes sensing surveys to include regions of scientific interest, these data have resulted in the targeting of three subglacial lakes for

exploration: SLV, in East Antarctica, and Subglacial Lake Ellsworth (SLE) and Subglacial Lake Whillans (SLW) beneath the West Antarctic Ice Sheet (WAIS; Figure 1.1).

Environmentally Responsible Exploration of Antarctic Subglacial Environments

Work at SLV showed that the use of a mechanical drill and a hydrocarbon based drilling fluid was a potential source of contamination to samples and the subglacial environment (e.g., Alekhina et al, 2007). Therefore, to meet mandates for environmental stewardship, the development of alternative, clean drilling and sampling technologies was necessary. The application of a hot water drill provided a means to address many environmental concerns.

Hot water drilling involves heating water sourced from melted snow to 80-90˚C, and using the thermal energy of the water to melt a hole though the ice mass. Melt water within the borehole is recirculated, reheated, and subsequently pumped to the drill head to continue the melting process. Since the "drilling fluid" used in this method is sourced from the ice overlying the subglacial lake, the introduction of contaminants is less of a concern given that the same ice naturally supplies water to the subglacial system. This method of drilling is also fast, allowing boreholes to be made in hours to days rather than over the course of several consecutive seasons (Makinson et al, 2016). Hot water drilling has been used to make boreholes as deep as 2.5km through Antarctic ice (Benson et al, 2014) and was successfully employed to gain access to Icelandic cauldera lakes in studies that showed microbial assemblages in the borehole water were not a source of contamination to the lake (Gaidos et al, 2004; Gaidos et al, 2009; Marteinsson et al, 2013).

Hot water drilling also provides the advantage of being amenable to modifications that can eliminate and reduce viable cells in the drill water (e.g., filtration and ultra violet irradiation; e.g., Doran et al, 2008). Such modifications were implemented and extensively tested by the Whillans Ice Stream Subglacial Access Research Drilling (WISSARD) project before accessing SLW. The WISSARD clean access

hot water drill included two large capacity filters (2.0µm and 0.2µm) and two UV light banks (185 and 245 nm) that were shown to be highly effective in removing and reducing the viability of cells, respectively (Priscu et al, 2013; Rack et al, 2014). A similar approach was to be used on the hot water drill designed to access SLE (Siegert et al, 2012).

The Attempt to Explore Subglacial Lake Ellsworth

SLE is a deep (~150m), narrow body of water (10km long and ~3km wide) that lies beneath the ~3km of ice of the WAIS (Siegert et al 2007; Figure 1.1). The lake was chosen as a site of exploration due to its proximity to existing infrastructure and its similarity to other subglacial lakes (e.g., size, temperature, and pressure; Siegert et al, 2007). To retrieve pristine water and sediment samples as well as collect real time data, engineers developed a single multipurpose probe and a sediment corer that were assembled and thoroughly decontaminated offsite (Siegert et al, 2012; Magiopoulos et al, 2016; Pearce et al, 2016). The stringent decontamination protocol for the probe and corer involved a combined treatment of surfaces with detergents, ethanol, commercial biocides, and hydrogen peroxide vapors, which were shown to reduce the level of bacterial and DNA contamination below the limits of detection (Magiopoulos et al, 2016; Pearce et al, 2016). During the 2012-2013 season, the Lake Ellsworth Consortium attempted to drill into SLE, but were unsuccessful due to a series of drilling, mechanical, and electrical complications that could not be overcome at the remote field site (Makinson et al, 2016; Pearce et al, 2016). The timeline for future drilling efforts has yet to be announced.

Exploration of Subglacial Lake Whillans

SLW is a small, active lake (maximum area of ~60 km^2) located along the Siple Coast of West Antarctica, beneath the ~800m thick ice of the Whillans Ice Stream (WIS; Fricker and Scambos, 2009; Christianson et al, 2012; Figure 1.1). Long term observations of the region have shown that water flows between several interconnected lake systems during episodic flooding events (Fricker and Scambos,

2009; Carter et al, 2013). SLW is one of the terminal lakes in this hydrologic network, receiving input from the upper WIS and the neighboring KIS (Smith et al, 2009; Wright and Siegert, 2012). Since 2003, SLW has flooded three times, with the outflow eventually making its way to the WIS grounding zone and draining into the Ross Sea (Fricker and Scambos, 2009; Siegfried et al, 2016). The short residence time of water in SLW (several decades; Fricker et. al., 2007), combined with its proximity to the United States research base, McMurdo Station, made it an ideal location to initiate subglacial lake exploration as any impact to the subglacial aquatic system should be minor and transitory (Fricker et al, 2011).

In 2013, the WISSARD project successfully implemented clean access hot water drilling technology and created a ~800m access borehole into the SLW water column. Upon breakthrough, lake water rose 28m into the borehole (Tulaczyk et al, 2014), indicating significant mixing of borehole with the lake water did not occur. At the time, SLW was at a low stand following a flooding event and had a water column depth of ~2.2m (Tulaczyk et al, 2014; Siegfried et al, 2016). Over the course of four days, water and shallow sediment cores were collected using a variety of approaches (Tulaczyk et al, 2014). Every instrument and sampling tool deployed into SLW was cleaned on site using 3% hydrogen peroxide and was subsequently passed through a germicidal UV light collar positioned at the mouth of the borehole to reduce microbial contamination of the lake (Priscu et al, 2013; Christner et al, 2014). The water and sediments recovered from SLW represent the only pristine samples collected from an Antarctic subglacial lake and provided the first opportunity to examine the biological, geochemical, and physical processes occurring in such environments.

Sub-Ice Shelf Environments

The stability of Antarctica's ice sheets, particularly the WAIS, is intimately connected to the integrity of its surrounding ice shelves (e.g., DeConto and Pollard, 2016). The ice shelves surrounding ~75% of the continent's coast are fed by terrestrially grounded ice and serve as buttresses, supporting

and controlling the movement of the land-based ice masses (Pritchard et al, 2012; Rignot et al, 2013). Consequently, the collapse of ice shelves has been attributed to accelerated terrestrial ice flow toward the continental margins and significant ice mass loss (De Angelis and Skvarca 2003; Scambos et al, 2004; Rignot et al, 2004; Rignot et al, 2013). The thinning and subsequent deterioration of ice shelves is accelerated as a result of basal and surficial melting due to warming atmospheric and ocean temperatures (Pritchard et al, 2012; Rignot et al, 2013; DeConto and Pollard, 2016). As ice shelves thin, their grounding lines (the transition point at which grounded ice begins to float) will retreat to more inland locations, displacing seawater (Goldberg et al, 2009). In West Antarctica, where most of the land mass is already below sea level, this retreat is expected to lead to the rapid breakdown of the ice sheet and have significant effects on global sea level rise (e.g., DeConto and Pollard, 2016).

Currently, ice shelves cover ~1.6 x10^6 km^2 of Antarctica's coast, an area equivalent to the size of the GRIS (Rignot et al, 2013) and yet, little is known about the ecosystems and communities that reside beneath them. The difficulty in accessing the marine cavities deep beneath ice sheets has largely limited studies to regions accessible from the ice shelf calving front; however, active biota (e.g., fish and crustaceans) have been observed as far as 400km from open ocean water (Lipps et al, 1979; Riddle et al, 2007; Post et al, 2014; Sugiyama et al, 2014). Studies following the collapse of the Larson B Ice Shelf revealed that benthic communities near the former ice margin were similar to that observed along the ice free continental shelf, while the community nearing the internal regions of the shelf was akin to communities found in deep sea environments (Rose et al, 2015).

Sub-ice shelf communities are thought to be dependent on the advection of nutrients and organic carbon from the open ocean (Riddle et al, 2007; Post et al, 2014; Sugiyama et al, 2014). However, there is evidence for sufficient chemosynthetic primary production to sustain higher trophic levels in the interior reaches of the Ross Ice Shelf (Horrigan et al, 1981). To date, investigations into the diversity and activity of microbial assemblages beneath ice shelves are extremely limited (Azam et al,

1979; Horrigan et al, 1981; Carr et al, 2013; Vick-Majors et al, 2015), preventing an adequate understanding of ecosystem function. Additionally, nothing is known about the influence of microorganisms and nutrients delivered by subglacial water and sediments to the sub-ice shelf cavities surrounding Antarctica (Carter and Fricker, 2012; Horgan et al, 2013).

Objective of Study

Over the last 20 years, research has shown that liquid water is abundant beneath the AIS in the form of a vast and complex hydrologic network. Studies of Arctic and Alpine glaciers suggest their subglacial aquatic environments harbor active microbial communities that influence the geochemical weathering of bedrock minerals and conduct nutrient cycling. However, there is a dearth of information on the diversity and activity of microorganisms inhabiting Antarctic subglacial environments. To address this deficit, this study examined the structure, diversity, and potential metabolic function of microbial communities that inhabit two distinct types of Antarctic sub-ice aquatic systems: (1) Subglacial Lake Whillans (SLW), an active lake beneath the Whillans Ice Stream (WIS); and (2) the WIS grounding zone, an important area representing the transition from a terrestrial to marine subglacial environment. Molecular data combined with concurrent analysis of the geochemistry of SLW made it possible to establish a conceptual model for how the subglacial lake ecosystem may persist in isolation from direct surface inputs. The SLW-WIS system is directly linked to the WIS grounding zone, allowing the opportunity to evaluate the physical, geochemical and microbiological contributions of this subglacial watershed on downstream habitats. Moreover, the first comprehensive study of a grounding zone ecosystem enables the establishment of a baseline with which to compare future ecological shifts associated with climate change and ice shelf collapse.

CHAPTER 2.
MICROBIAL COMMUNITY STRUCTURE OF SUBGLACIAL LAKE WHILLANS, WEST ANTARCTICA

Introduction

Remote sensing and field surveys have identified ~400 lakes beneath the Antarctic ice sheet (Smith et al, 2009; Wright and Siegert, 2012), provided evidence for the widespread occurrence of water-saturated sediments (e.g., Studinger et al, 2001), and demonstrated hydrologic connections between certain lakes (Fricker et al, 2007). The abundance of water and availability of metabolic energy sources beneath the ice sheet (Bell, 2008; Smith et al, 2009; Skidmore 2011; Palmer et al, 2013) supports the possibility for microbial ecosystems and has motivated international exploration efforts in West and East Antarctica (Fricker et al, 2011; Lukin and Bulat 2011; Ross et al, 2011). Although little information has been available to infer conditions in Antarctica's vast and complex subglacial aquifer, microbial cells inhabiting this biome are estimated at ~10^{29} (Priscu et al, 2008); a value similar to global estimates for open ocean waters (Whitman et al, 1998). Fortunately, recent progress in subglacial exploration (Tulaczyk et al, 2014) has provided an opportunity to directly examine the nature, evolution, and biogeochemical contributions of microbial communities at the bed of Antarctica's ice sheet.

Much of the data amassed on subglacial microbial communities has been derived through analyses of subglacial water outflows from alpine and polar glaciers (Skidmore et al, 2005; Cheng and Foght, 2007; Mikucki and Priscu, 2007; Boyd et al, 2011; Hamilton et al, 2013; Dieser et al, 2014) or basal materials retrieved from deep ice boreholes (e.g., Priscu et al, 1999; Lanoil et al, 2009; Christner et al, 2012). This approach has been valuable for providing baseline data and identifying common trends in disparate subglacial ecosystems. For example, there is geochemical and microbiological evidence that subglacial microbes derive their energy through the weathering of bedrock minerals and thus influence subglacial water chemistry (Skidmore 2011; Mitchell et al, 2013). The prevalence of phylotypes related to species of *Thiobacillus* and *Sideroxydans* suggests primary production at the glacier bed may rely on reduced iron and sulfur compounds liberated through glacial comminution and microbiological

processes occurring in the sediments or at the bedrock interface (Skidmore et al, 2010; Boyd et al, 2014). Evidence for the activity of methanogenic, methanotrophic, and ammonia oxidizing species has also been provided in several subglacial environments (Boyd et al, 2010; Boyd et al, 2011; Dieser et al, 2014), implying these pathways could also play important roles in carbon and nitrogen cycling beneath larger ice masses. Although these pioneering efforts have provided valuable data to generate hypotheses on the structure and function of subglacial microbial ecosystems, their applicability to environments beneath ice sheets has remained uncertain.

Directly sampling sub-ice aquatic environments in a microbiologically-clean manner is logistically challenging (Doran et al, 2008; Siegert et al, 2012), requiring strategies to reduce microbial cells associated with the drilling process and minimize exchange between the surface and subglacial environment (Priscu et al, 2013). During January 2013, the Whillans Ice Stream Subglacial Access Research Drilling (WISSARD) Project conducted the first successful sampling of an Antarctic subglacial lake (Christner et al, 2014; Tulaczyk et al, 2014). Christner et al. (2014) reported that planktonic bacteria and archaea in the aerobic water column were at an average concentration of 1.3×10^5 cells mL^{-1} and morphologically diverse. Molecular analysis of 16S rRNA gene sequences amplified from the water column and surficial sediments (0-2cm) revealed a rich prokaryotic community consisting of several phylotypes similar to chemosynthetic species that have been observed in alpine and polar glacier environments (e.g. members of *Thiobacillus*, *Sideroxydans*, and *Methylobacter*; Lanoil et al, 2009; Boyd et al, 2014; Dieser et al, 2014). Furthermore, primary and heterotrophic production data revealed that SLW contained a metabolically functional microbial community that may be sustained by dark autotrophic activity (Christner et al, 2014).

Here we present a detailed description of SLW's water column and sediment (to depths of 36 cm) communities based on analysis of amplified 16S rRNA genes (rDNA) and molecules (rRNA). This approach served the dual role of providing information on microbial community structures while also

allowing an assessment of potentially metabolically active taxa and the biogeochemical reactions they are likely to catalyze. Our data provide an initial framework for discerning the diversity and ecology of Antarctic subglacial lake environments, and support the hypotheses that microbial transformations beneath ice masses are driven by chemosynthesis and have global biogeochemical significance (Wadham et al, 2012).

Materials and Methods

Site Description and Drilling Operations

SLW is centrally located in the lower Whillans Ice Stream (WIS), West Antarctica beneath ~800m of ice and has a maximum area of ~60 km^2. The water column depth was ~2.2 m when sampled in January 2013 (Fricker and Scambos, 2009; Christianson et al, 2012; Tulaczyk et al, 2014). Observations of ice surface elevation changes in this region of the WIS have provided data to infer subglacial hydrological conditions and examine their influence on ice sheet behavior (Bell, 2008; Pritchard et al, 2012; Carter et al, 2013). SLW was shown to receive episodic water input from the upper WIS and the neighboring Kamb Ice Stream, and as such, is classified as an active lake (Smith et al, 2009; Wright and Siegert, 2012). Since 2003, SLW has filled and drained three times (Siegfried et al, 2016). The outflow is transported via subglacial channels ~100 km to the grounding zone and drains into the marine cavity beneath the Ross Ice Shelf (Fricker and Scambos, 2009). At the time of sampling, SLW was at a low stand and filling (Siegfried et al, 2016). The surface sediments of the lake bed were composed primarily of glacial till that was likely deposited during flood events (Hodson et al, 2016), while the deeper sediments contained seawater signatures that linked their origin to past marine intrusions in the region (Christner et al, 2014; Michaud et al, 2016).

During January 2013, a hot water drilling system (Blythe et al, 2014; Burnett et al, 2014; Rack et al, 2014) was used to create a ~0.6 m diameter borehole (location 84.240˚ S, 153.694˚W) in the ice overlying SLW. The borehole provided direct access to the SLW water column and underlying sediments

over a period of 3 days. Details on the scientific operations conducted by WISSARD at SLW are provided in Tulaczyk et al. (2014). Multi-sample analysis of the circulating water was conducted during drilling to assess the microbial assemblages present. The sampling procedure and results of this analysis are presented in the supplemental material.

Water Column and Sediment Sampling

Particulates in the SLW water column were concentrated by filtration *in situ* using a Water Transfer System- Large Volume (WTS-LV; McLane Research Laboratories Inc.) that was modified for borehole deployment. During 68 h of borehole operations, the WTS-LV was deployed three times (Tulaczyk et al, 2014). Each cast was made at the approximate middle of the 2.2m water column and filtration occurred for ~2 h. Particulates in the water were collected using a custom, modular 142 mm PVC filter holder and sequentially concentrated on Supor membrane filters (Pall Corp.) with pore sizes of 10, 3, 0.8, and 0.2 µm. Flow was measured with an analog meter on the WTS-LV, and 4.9, 5.3, and 7.2 L of water was concentrated during the first, second, and third casts, respectively. Upon retrieval from the borehole, the filter housing was transferred to a class 100 laminar flow hood (Labconco). The filters were removed from the housing, placed in sterile 142 mm petri dishes, and quartered with a sterile scalpel. Each quarter was placed in a 7 mL cryovial and preserved by the addition of 5 mL of RNAlater (Ambion) or DNA preservation solution (40mM EDTA pH 8.0, 50mM Tris pH 8.3, 0.73M sucrose). Filters amended with RNAlater were incubated at 4˚C for 10-12 h before freezing. All samples were stored at -80^{o}C until analyzed.

A 36 cm long sediment core was collected using a multicoring device (Uwitec). The core was sectioned at 2 cm intervals in a class 100 laminar flow hood (Labconco), and 7-15 g of each interval was placed in sterile 60 mL Nalgene bottles, preserved in either RNAlater or DNA preservation solution, and mixed prior to storage at -80^{o}C. The procedures used for the extraction, amplification and sequencing of nucleic acids are documented in the supplemental methods (Appendix A).

Phylogenetic and Statistical Analysis

Paired end sequence reads from the V4 region of the 16S rRNA gene (Caporaso et al, 2012) were assembled into contigs, quality filtered using the Mothur phylogenetic analysis pipeline (v1.33.3; Schloss et al, 2009), and aligned with the Mothur-compatible version of the SILVA database (v119). Chimeric sequences were identified and removed using the Uchime algorithm (Edgar et al, 2011), as implemented within Mothur. All sequences with ≥97% 16S rRNA gene sequence similarity were defined as an operational taxonomic unit (OTU) and classified using the SEED database (v119; Pruesse et al, 2012). The taxonomic classification of representative sequence from each OTU were further evaluated through classification with the NCBI GenBank database. Diversity and richness estimations (Shannon, Inverse Simpson, and Chao1) were calculated using Mothur on OTU abundance data normalized to the smallest sample dataset (25,904 sequences). Singletons were removed before further analysis. Abundant OTUs were defined as having a relative abundance ≥1% of the sequences from a given sample. All abundant OTUs in drilling and extraction control samples were designated as potential contaminants. Nonmetric multidimensional scaling (NMDS) plots were computed within R (v.3.1.2; RC Team, 2015) on logarithmically transformed data and Bray Curtis dissimilarity matrices using the Vegan (Oksanen et al, 2013) and Phyloseq (McMurdie and Holmes, 2013) packages. Sequence data are available in the NCBI Sequence Read Archive under project PRJNA244335.

Results

Composition of the Abundant Bacterial and Archaeal OTUs in SLW

The OTU composition and abundances in samples from the water column were significantly different from those of the sediment ($p \leq 0.005$), with the surficial horizon (0-2 cm) showing the highest similarity to the lake water (Figure 2.1). Both the lake water and sediment communities were statistically distinct from those in the drill and borehole water ($p \leq 0.008$), and grouped distinctly based on NMDS analysis (Figure 2.1). A detailed description of the microbial assemblages found in the drill

system, borehole, and methodological controls are provided in the supplemental material (Appendix A). Overall, the SLW microbial community structures derived through the analysis of rRNA or rDNA sequences were not statistically different (p=0.12).

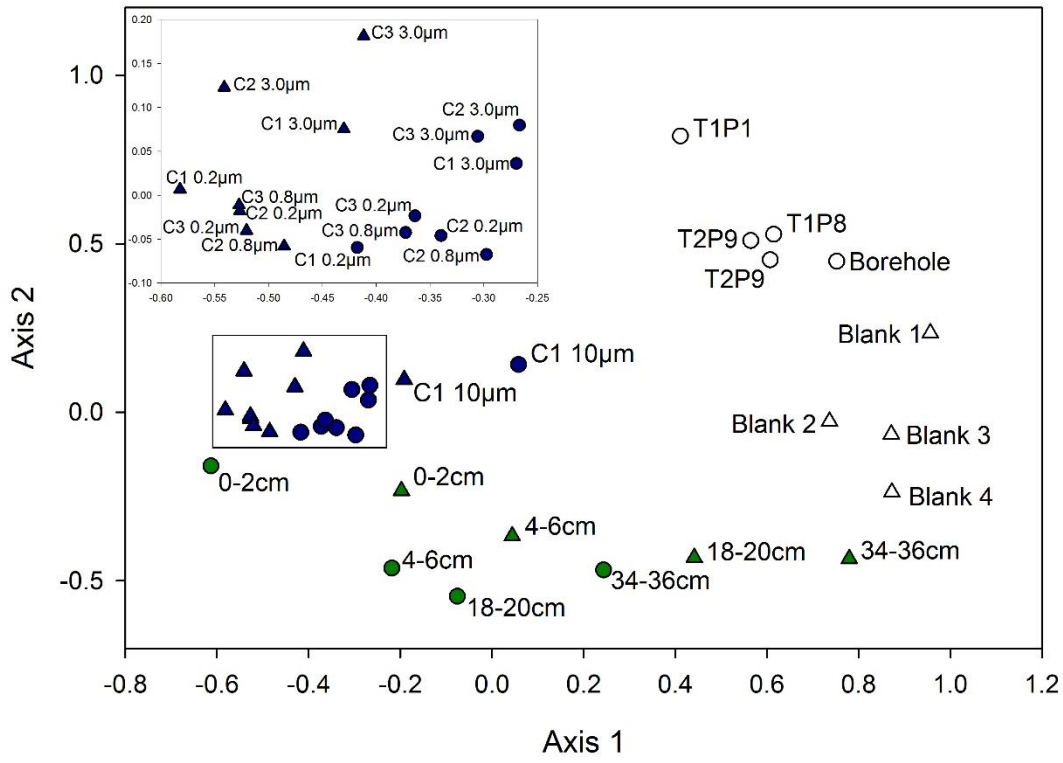


Figure 2.1. Nonmetric multidimensional scaling plot based on OTUs classified from the 16S rRNA and rRNA gene sequences obtained from samples of the drilling water, SLW water column, sediments, and experimental controls. Circles represent rDNA based sample libraries and triangles represent rRNA based libraries. Lake water is denoted by blue symbols, sediments are green, and controls are white. Inset contains the 3.0, 0.8, and 0.2 um samples from each water cast outlined with a black box.

The vast majority of sequences obtained from the SLW water column and sediments could be taxonomically assigned at the domain level (<0.2% were unclassified). The SLW water column was dominated by bacteria, with only 3% and 2% of the OTUs classifying as Archaea in the rDNA and rRNA libraries, respectively, whereas <0.3% of the sediment community was archaeal. The phylum *Thaumarchaeota* comprised ~98% of archaeal OTUs within the water column rRNA libraries, while *Euryarchaeaota* represented 85% of archaeal sequences obtained from the sediments. The most abundant archaeal OTU in SLW (OTU000074), comprising 2% of the rDNA and 0.9% of the rRNA-based

sequences, classified within the genus *Candidatus* Nitrosoarcheaum (Figure 2.2). The most abundant Euryarchaeota OTU in the rRNA libraries is most closely related to *Methanohalophilus levihalophilus* (91% sequence identity) and was only detected in the 34-36cm sediment horizon.

Proteobacteria were the dominant phylum in the SLW ecosystem. Water and surficial sediments (0-2 cm) were primarily comprised of *Betaproteobacteria*, while the abundance of *Gammaproteobacteria* generally increased with sediment depth (Supplemental Figure A.2). The most abundant OTU (OTU000528; ~8% rRNA) within the water column community was closely related to *Polaromonas glacialis* (100% identity; Figure 2.2; Supplemental Table A.2). Many of the OTUs abundant in the lake were rare (<0.01%) in the sediment community, including taxa most closely related to species of *Ferriphaselus* (96% identity; OTU000051) and *Solitalea* (94%; OTU000093), as well as several poorly classified members of the *Proteobacteria* and *Lentisphaerae* (Figure 2.2). Also prevalent in the lake were several OTUs (OTUs 000073, 000118, and 022091) sharing ≥ 98% identity to *Candidatus* Nitrotoga arctica (Figure 2.2; Supplemental Table A.2), representing 9 and 13% of the rRNA and rDNA water sequences, respectively. This taxa was also found in the sediment community (2% in rRNA and 5% in rDNA at 0-2cm), but their abundance decreased rapidly with depth (Figure 2.2).

Phylotypes in the genera *Albidiferax, Sideroxydans, Thiobacillus, and Methylobacter* were shared between the water column and surficial sediments (0-2cm). An OTU (000038) having 99% identity with *Sideroxydans lithotrophicus* was the third most abundant OTU in both the water column and 0-2cm sediment horizon (rRNA; Figure 2.2). Two OTUs (002673 and 003072) closely related to *Albidiferax ferrireducens* (≥99% Identity), were also highly prevalent in the water column (5% of rRNA and 3% rDNA sequences) and sediments (~1% of the rRNA and DNA sequences; Supplemental Table A.2). The *Albidiferax*-related OTUs also showed a preference based on size, with larger representation in the 10 and 3 µm rRNA libraries (Figure 2.2). *Methylobacter*- (OTU000112) and *Thiobacillus*-related

(OTU000064) taxa were also present in the water column rRNA libraries (2 and 1%, respectively), but their abundance was ~10-fold higher in the surficial sediments (0-2 cm; Figure 2.2).

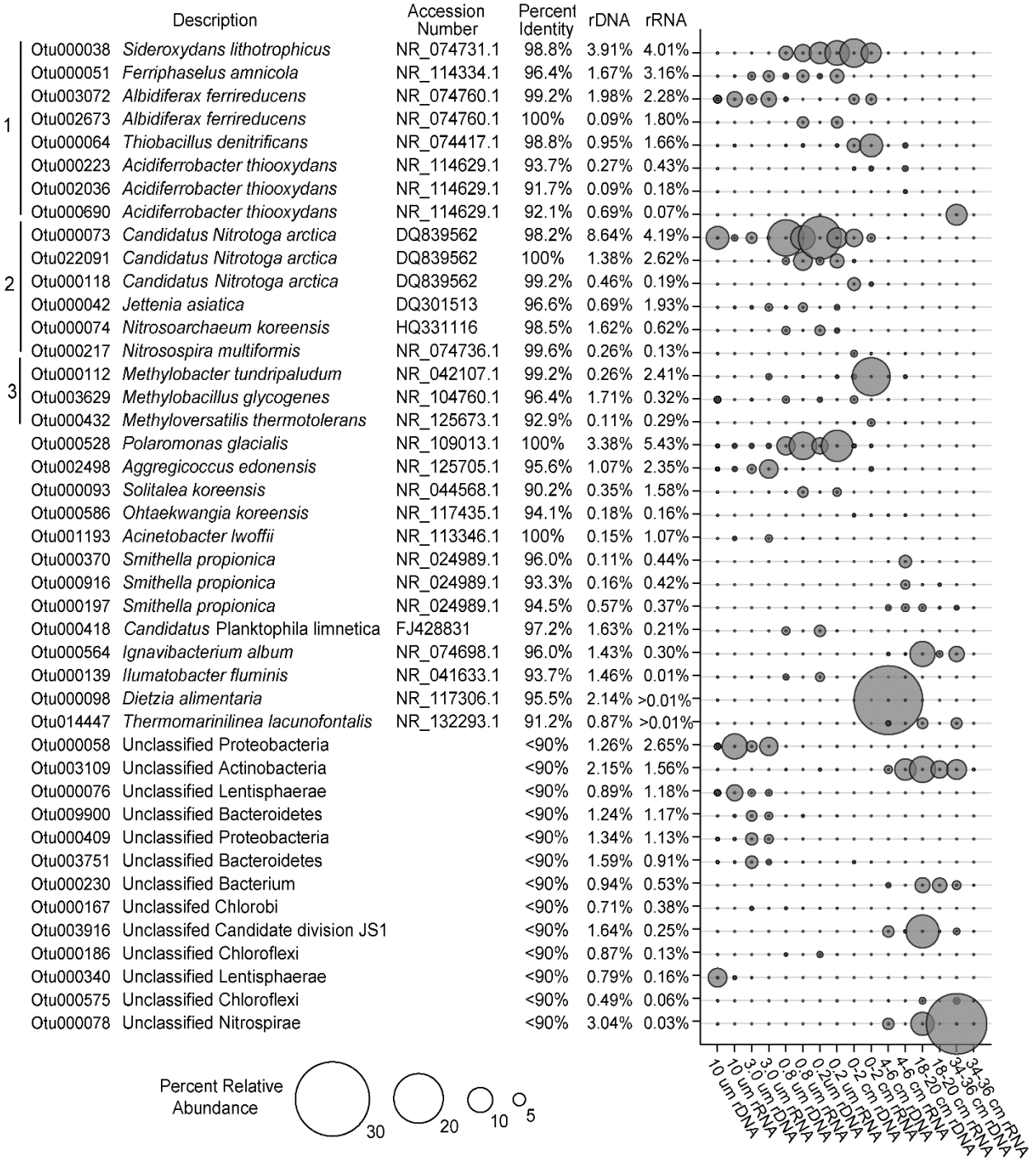


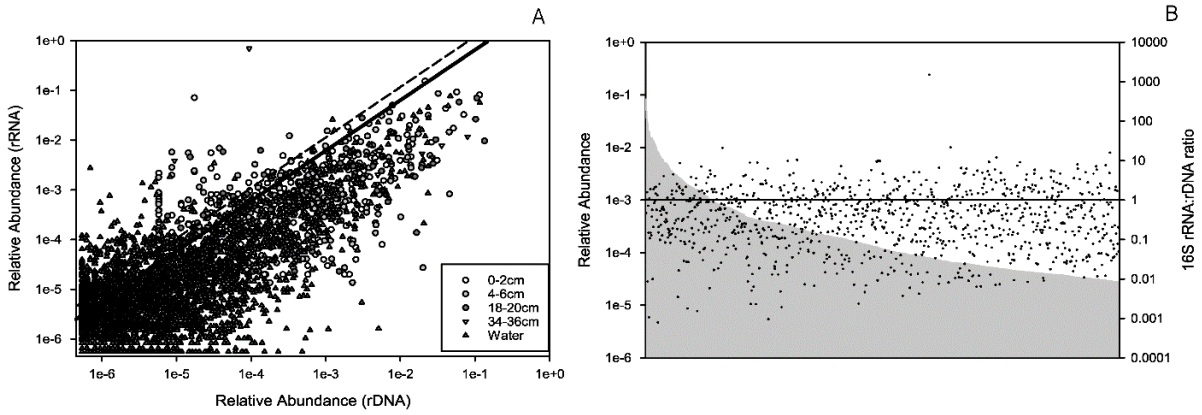
Figure 2.2. The most abundant OTUs in the bulk SLW water column and sediments (>1%). All of the OTUs are listed with their nearest taxonomic neighbor and total relative abundance in the overall SLW community. Brackets 1, 2, and 3 represent putative taxa that are involved in sulfur/iron, nitrogen, and methane cycling, respectively.

At sediment depths between 4 and 36 cm, several abundant OTUs in the rRNA libraries (OTUs 000197, 000370, and 000916) most closely related to *Smithella propionica* (≥ 93% identity) comprised 4% of the sediment rRNA sequences (Figure 2.2). Also abundant in the deeper horizons (>6 cm) was an OTU (000564) 96% identical to *Ignavibacterium album* and one that classified as a member of the Candidate division JS1 (OTU003961; Figure 2.2). When compared to the water column, the Candidate division JS1, *Nitrospirae*, and *Gammaproteobacteria* were generally higher in abundance in the sediments (Supplemental Figure A.2).

16S rRNA: rDNA Ratios

The ratio of 16S rRNA to rDNA sequence abundance for each OTU identified in SLW was examined. Of the OTUs detected in both the rRNA and rDNA based libraries, 59% had ratios greater than one, and there was a positive correlation (Pearson Correlation; r = 0.77) between the abundance of rRNA and rDNA sequences for individual OTUs (Figure 2.3A). No correlation was observed between the relative abundance of OTUs and their rRNA:rDNA ratios (Figure 2.3B).

The relative abundance of 16S rRNA to rDNA sequences for a given OTU can be an indication of their potential for metabolic activity (Blazewicz et al, 2013); therefore, OTUs with the largest ratios (≥5.8; 99th percentile) were examined in greater detail. Several of the numerically abundant phylotypes (e.g., species of *Albidiferax*, *Methylobacter, Candidatus* Nitrotoga*, Sideroxydans, and Smithella*) were also in the top 1% of the community based on high rRNA:rDNA ratios (Figure 2.3C; Supplemental Table A.3). Rarer taxa, such as those closely related to *Desulfatiglans parachlorophenolica* (95% identity; OTU000609) and a poorly classified member of the *Chloroflexi* (OTU005695), were among the OTUs with the largest rRNA:rDNA abundance ratios observed (38 and 18, respectively).



		Description	Accession Number	Percent Identity	rDNA	rRNA
1	Otu002673	*Albidiferax ferrireducens*	NR_074760.1	100%	0.08%	1.67%
	Otu000609	*Desulfatiglans parachlorophenolica*	NR_126176.1	95.3%	0.03%	0.31%
	Otu000380	*Sideroxydans lithotrophicus*	NR_074731.1	97.6%	0.01%	0.12%
	Otu000690	*Acidiferrobacter thiooxydans*	NR_114629.1	92.1%	0.70%	0.07%
	Otu013742	*Desulfatibacillum alkenivorans*	NR_074962.1	96.8%	<0.01%	0.02%
	Otu022106	*Ectothiorhodospira mobilis*	NR_125567.1	92.5%	0.01%	0.02%
2	Otu000279	*Candidatus* Nitrotoga arctica	DQ839562	98.4%	0.08%	0.15%
	Otu002533	*Candidatus* Nitrotoga arctica	DQ839562	99.1%	0.01%	0.12%
	Otu000583	*Brocadia fulgida*	LAQJ01000287	94.2%	0.09%	0.09%
3	Otu000112	*Methylobacter tundripaludum*	NR_042107.1	99.2%	0.26%	2.24%
	Otu002498	*Aggregicoccus edonensis*	NR_125705.1	95.3%	1.01%	2.18%
	Otu001193	*Acinetobacter lwoffii*	NR_113346.1	100%	0.14%	0.99%
	Otu000370	*Smithella propionica*	NR_024989.1	96.0%	0.11%	0.41%
	Otu000733	*Byssovorax cruenta*	NR_042341.1	90.1%	0.05%	0.40%
	Otu000221	*Sphingomonas aquatilis*	NR_113867.1	100%	0.03%	0.30%
	Otu002195	*Solitalea canadensis*	NR_074099.1	91.7%	0.04%	0.24%
	Otu001065	*Erythrobacter pelagi*	NR_117936.1	100%	0.02%	0.14%
	Otu010260	*Syntrophus aciditrophicus*	NR_102776.1	95.7%	0.04%	0.06%
	Otu008210	*Lautropia mirabilis*	NR_104897.1	96.0%	0.01%	0.03%
	Otu025293	*Labilithrix luteola*	NR_126182.1	95.3%	<0.01%	0.03%
	Otu000307	*Smithella propionica*	NR_024989.1	92.9%	<0.01%	0.02%
	Otu006794	*Tabrizicola aquatica*	NR_117979.1	98.8%	<0.01%	0.02%
	Otu001312	Unclassified Chlorobi		<90%	0.04%	0.10%
	Otu005695	Unclassified Chloroflexi		<90%	0.02%	0.07%
	Otu015663	Unclassified Bacteria		<90%	0.01%	0.02%
	Otu016841	Unclassified Bacteria		<90%	<0.01%	0.02%
	Otu024095	Unclassified Armatimonadetes		<90%	0.01%	0.01%
	Otu034306	Unclassified Bacteria		<90%	0.00%	0.01%

Figure 2.3. The ratio of 16S rRNA to rDNA sequences obtained from the SLW molecular data. (A) The relative abundance of individual OTUs in rRNA versus the rDNA sequence data. The black line denotes those OTUs in the top 1% (ratio of 5.8) while those above the hashed line represent outliers (ratio >10.7). (B) The rank abundance of OTUs, based on 16S rDNA data, and their rRNA:rDNA ratios denoted as black dots. The horizontal black line denotes an rRNA:rDNA ratio of 1. (C) List of the top 1% of OTUs with rRNA:rDNA ratios ≥5.8, identified by their nearest taxonomic neighbor. Bracket designations are the same as Figure 2.2.

Discussion

Antarctic subglacial lakes have been a scientific curiosity since their discovery (Oswald & DeRobin, 1973). The initial studies on accretion ice from Subglacial Lake Vostok concluded that viable microbes were present in the surface waters of the lake (Karl et al, 1999; Priscu et al, 1999), but due to concerns about sample contamination (e.g., Christner et al, 2005), evidence supporting the habitability of Antarctica's subglacial environment was subject to criticism. More recently, studies have demonstrated the suitability of subglacial environments as ecosystems and shown that liquid water reservoirs at the base of glaciers harbor diverse assemblages of microorganisms (e.g., Skidmore et al, 2005; Boyd et al, 2011; Hamilton et al, 2013; Dieser et al, 2014). However, progress in understanding Antarctic subglacial lake microbiology has been hampered by logistical constraints that make sampling and direct observations challenging. The WISSARD project collected the first pristine water and sediment samples from an Antarctic subglacial lake (Christner et al, 2014), providing an unprecedented opportunity to examine microbial community structure and infer ecosystem processes in an aquatic habitat beneath the West Antarctic Ice Sheet (WAIS).

Conclusions on the composition of SLW microbial communities can be made with confidence given the significant differences they displayed from assemblages in the drilling water and procedural controls (Figure 2.1; Supplementary Results). With the exception of *Janthinobacterium, Tumebacillus* and *Herbaspirillium*-related OTUs, OTUs identified in the drilling water were rare or absent in lake water samples. Microorganisms that were present in the borehole and drill water may be human-based contaminants, derived from the drill system plumbing, and/or have originated from the snow and glacial ice that sourced the water for the drill (Blythe et al, 2014; Rack et al, 2014). The latter is supported by the abundance of OTUs related to *Tumebacillus* and *Janthinobacterium* in drilling and borehole water, as these taxa have been frequently identified in polar environments (e.g., Steven et al, 2008; Kim et al, 2012). Alternatively, the presence of OTUs related to *Herbaspirillum*, a bacterial genus that frequently

contaminates DNA extraction kits (Salter et al, 2014), may be an artifact of laboratory contamination. Determining the conclusive source of the *Herbaspirillum* phylotypes is complicated by the fact that limnological studies of surface Antarctic lakes have identified these taxa as bona fide members of the bacterioplankton communities (Pearce et al, 2003; Kuhn et al, 2014). Given these uncertainties, we applied a conservative approach to the data analysis and eliminated all of the OTUs discussed above from the SLW community descriptions.

In the absence of light and photosynthetic activity, chemolithoautotrophic species may play a crucial role in carbon cycling through the generation of new organic carbon for the subglacial ecosystem. Christner et al. (2014) concluded that rates of CO_2 fixation in samples from the SLW water column were sufficient to support heterotrophic production. Given that the waters of SLW are predominantly derived from glacial melt, the bulk of nutrients involved in biogeochemical cycling are likely sourced from subglacial sediments that contain relict marine organic matter and are widespread beneath this portion of the WAIS (Wadham et al, 2012; Christner et al, 2014). Hence, the underlying geology and historical connection to the Ross Sea was expected to strongly influence the structure and metabolic function of microorganisms inhabiting the SLW ecosystem.

Similar to other subglacial environments (Christner et al, 2001; Skidmore et al, 2005; Lanoil et al, 2009; Hamilton et al, 2013; Dieser et al, 2014), the microbial species of SLW were largely composed of *Proteobacteria*, *Actinobacteria*, *Bacteriodetes* and *Firmicutes* (Supplemental Figure A.2), and also contained members of the *Nitrospirae*, *Chloroflexi*, *Chlorobi, Thaumarchaeota*, and Candidate Division JS1 phyla (Supplemental Figure A.2). The most abundant taxa in SLW (*Polaromonas*, *Sideroxydans*, and *Thiobacillus*) were closely related to sequences characterized from sediment cores recovered from beneath the neighboring Kamb Ice Stream (Lanoil et al, 2009), while *Methylobacter*, *Albidiferax, Candidatus* Nitrotoga, and *Thaumarchaeota* species have been shown to be prevalent in subglacial outflows, basal ice, permafrost, and polar waters (Alawi et al, 2007; Cheng and Foght 2007; Alonso-Saez

et al, 2011; Dieser et al, 2014; Doyle, 2015). The widespread distribution of these taxa in cold environments suggests an inherent tolerance of conditions in the cryosphere, and that they may serve important ecological functions in the subglacial habitat.

The relationship between abundances of sequenced 16S rRNA molecules and genes has been used as a means to assess the metabolic activity of individual taxa (e.g., DeAngelis et al, 2010; Jones and Lennon 2010; Campbell et al, 2011; Dieser et al, 2014). Although cellular ribosome concentration correlates positively with growth rate, the interpretation of rRNA:rDNA ratios in natural communities is more complex because the relationship is not uniform for all species (Blazewicz et al, 2013) and may require a fundamentally different interpretation for oligotrophic versus copiotrophic lifestyles (Lankiewicz et al, 2015). In light of these uncertainties, high 16S rRNA abundance and rRNA:rDNA ratios were evaluated to specifically identify outliers in the SLW community data. The OTUs identified through this analysis are inferred to have the greatest potential for metabolic activity within the community and thus most likely to be influencing biogeochemical transformations in SLW at the time it was sampled. The positive correlation between the abundance of rRNA and rDNA in the SLW OTUs (Figure 2.3A) supported that taxa abundant in the rDNA data were ecologically important and unlikely to represent dead cell populations. Rank abundance of the taxa did not correlate with the rRNA:rDNA ratio (Figure 2.3B), suggesting that abundance per se was not a good predictor for potential metabolic activity. Although assumptions about metabolic function based on short 16S rRNA gene fragments can be tenuous, many of the numerically abundant (Figure 2.2) and rare OTUs (Figure 2.3C) with large rRNA:rDNA ratios (>5.8; Figure 2.3C) were phylogenetically most closely related to bacterial and archaeal species that use reduced nitrogen, iron, and sulfur compounds or C1 compounds as primary electron donors (Figure 2.4).

Geochemical analysis of the dissolved inorganic nitrogen pool in the SLW water column showed that it was comprised primarily of ammonium (~2.4 μmol L^{-1}) and nitrate (0.8 μmol L^{-1}), with $\Delta^{17}O\text{-}NO_3^-$

values (-0.1‰ to 0.2‰) indicating *in situ* nitrification (Christner et al, 2014). The potential for nitrification was also supported by the abundance of taxa classifying within the Thaumarchaeaota (Supplemental Figure A.2 and Figure 2.3), a ubiquitous clade containing chemolithotrophic (Könneke et al, 2005) and mixotrophic (Qin et al, 2014) archaea that derive energy from ammonia oxidation.

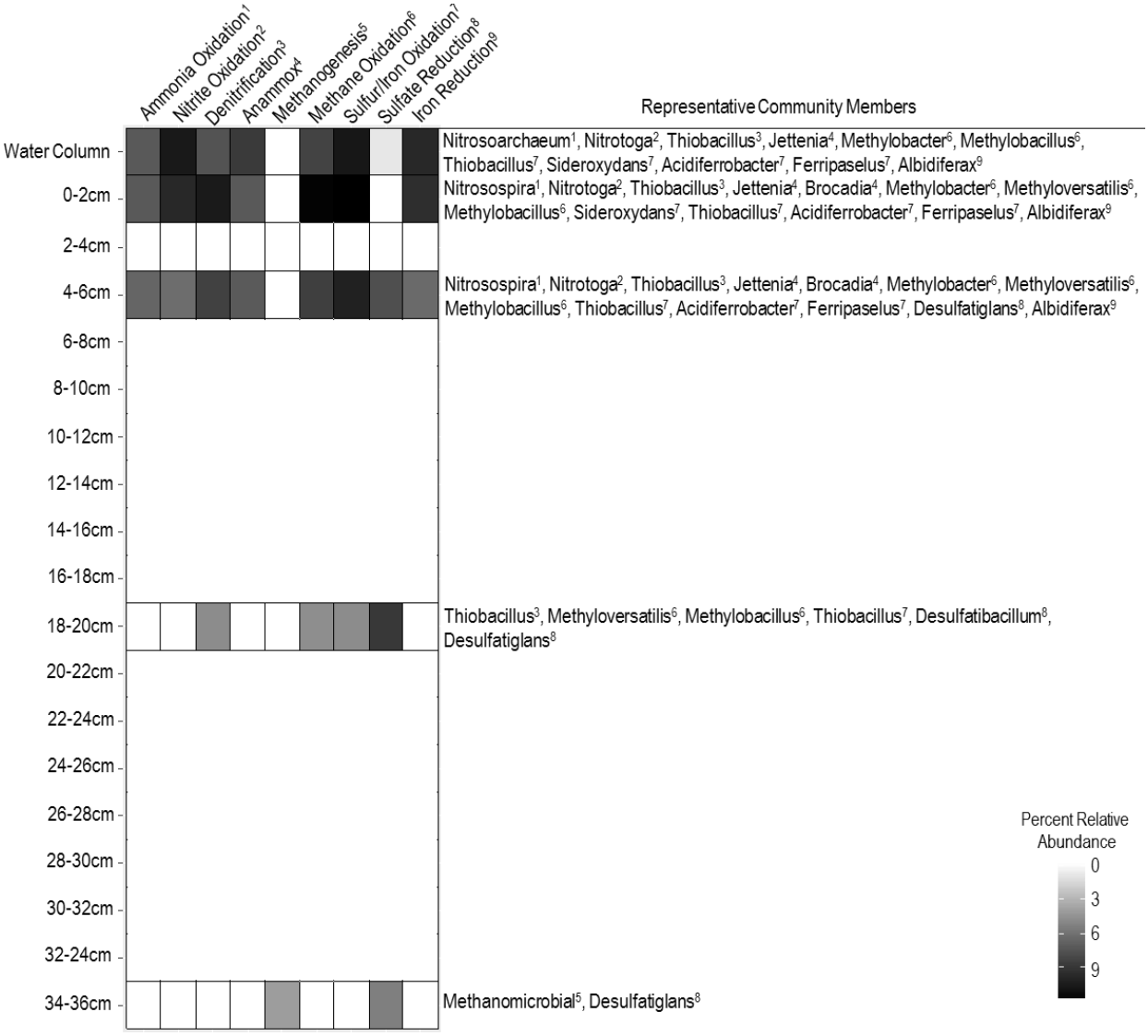


Figure 2.4. Heat plot showing the occurrence of various potential metabolic functions in SLW based on 16S rRNA abundances of important community members.

Water column OTUs related to the ammonia oxidizing archaea (AOA) were predominately found as cell populations that passed through 3.0 µm pores and were primarily retained on the 0.8 and 0.2 µm filters (Figure 2.2), which agreed well with individual cell sizes reported for this group (<1µm Könneke et al,

2005). The AOA were 1.6- and 14-times (rRNA and rDNA, respectively) more abundant than known ammonia oxidizing bacterial (AOB) genera in the water column; however, the abundance of AOA quickly diminished with sediment depth, while the AOB increased (55-fold higher in 0-2cm rRNA, Figures 2.2 and 2.4). The most abundant AOB-related OTU in the sediments had 99% sequence identity with *Nitrosospira multiformis* (Figure 2.2). AOA have been found to outcompete AOB in environments with low ammonium concentrations (<1 μM), presumably due to the higher affinity of their ammonia monooxygenase for substrate (Martens-Habbena et al, 2009). This fact may explain the distribution of AOA and AOB in SLW, as ammonium was ~40-times more abundant in sediment pore waters than in the lake (Vick-Majors, 2016). Further, the water column and sediments (0-18 cm) contained abundant OTUs and rare OTUs with large rRNA:rDNA ratios that were related to *Candidatus* Jettenia asiatica (OTU000042; Figure 2.2) and *Brocadia fulgida* (OTU000583; Figure 2.3C); taxa of anaerobic ammonia oxidizing bacteria (Kartal et al, 2008; Hu et al, 2012; Figure 2.4). In the absence of oxygen, these microorganisms may serve an important role in nitrogen removal within the sediments or during conditions of water column hypoxia.

With only one known exception (i.e., van Kessel et al, 2015), the second step of nitrification is catalyzed by a physiologically distinct group of microorganisms that oxidize nitrite to nitrate. Based on the SLW water column data, we conclude that this reaction is probably carried out by a group of Betaproteobacteria closely related to *Candidatus* Nitrotoga arctica, a nitrite-oxidizing isolate from Siberian permafrost (Alawi et al, 2007; Figure 2.4). OTUs related to *Candidatus* Nitrotoga were highly abundant in the water column (~10% rRNA) and surficial sediment (0-2cm; 3.8% rRNA; Figure 2.4). Also prevalent within the surficial sediments (0-6cm) were OTUs closely related to *Thiobacillus dentrificans* (99% sequence identity; Figure 2.2 and 2.4), a microorganism with the capacity to use inorganic sulfur compounds or Fe^{2+} as an electron donor via aerobic respiration or denitrification (Beller et al, 2006).

Studies of subglacial sediments from alpine glaciers (e.g., Skidmore et al, 2005; Boyd et al, 2014; Harrold et al, 2015) and the WAIS (Lanoil et al, 2009) have provided evidence that sulfide and iron oxidation are microbially-mediated weathering processes that occur at the bed. Boyd et al. (2014) demonstrated that microbial pyrite oxidation was an important weathering reaction beneath the Robertson Glacier that may contribute to subglacial primary production. Within the water and surficial sediments of SLW, species of *Sideroxydans* and relatives of the iron-oxidizer *Ferriphaselus amnicola* (Figure 2.2, 2.3 and 2.4; Kato et al, 2014) could participate in a similar process. Taxa closely related to *Albidiferax ferrireducens* may also contribute to the redox cycling of iron under certain conditions. *Albidiferax ferrireducens* is a heterotrophic, facultative anaerobe capable of coupling the reduction of Fe^{+3} to the oxidation of organic compounds such as acetate (Finneran et al, 2003), which was ~1 µM in the SLW water column (Christner et al, 2014; Figure 2.4). *Albidiferax*-related OTUs were found within the surficial sediments, but in the water column, the majority were observed on the 10 and 3.0 µm filter size fractions, consistent with the size reported for the type strain (3-5 µm filaments; Finneran et al, 2003) and/or attachment to water column suspensoids. It is notable that members of this genera have also been found abundant in other glacier ecosystems, including basal ice facies from the Matanuska Glacier, Alaska and periglacial streams near Thule, Greenland (Doyle, 2015).

Sequences related to the adenosine-5'-phosphosulfate reductase (*aprA*) gene, a key enzyme involved in sulfur redox chemistry, of *Sideroxydans* and *Thiobacillus* species were detected in SLW sediments (Purcell et al, 2014). Purcell et al. (2014) used quantitative PCR and estimated 0.037 to 9.5 $x10^5$ *aprA* gene copies per gram of wet SLW sediment, which represented from 0.9% (28-34cm) to 15% (0-4cm) of the 16S rRNA gene copies that were also detected. This is consistent with our rDNA analysis which showed that the *Sideroxydans*- and *Thiobacillus*-related OTUs accounted for 12% of sequences in the 0-2cm sediment horizon, decreasing to 0.01% at a depth of 34-36cm (Figure 2.2). Members of these genera were also inferred to be highly abundant in the SLW water column (OTUs000038 and 000064;

Figure 2.2) and had large rRNA:rDNA ratios (OTU000380 ; Figure 2.3C and 2.4). Purcell et al. (2014) also measured low rates of sulfate reduction in laboratory experiments with SLW sediments. Known sulfate reducing bacteria were rare in the SLW sediment community and sulfate profiles in the sediment porewaters suggested sulfate reduction was not an active process in the upper 36 cm of the sediments (Michaud et al, 2016). However, OTUs related to the genera *Desulfatiglans* and *Desulfatibacillum* had high rRNA:rDNA ratios in the 4-6cm and 16-18cm sediment horizons, consistent with their potential to conduct sulfate reduction in the SLW sediments when conditions are favorable (Figure 2.4).

The basal sediments beneath ice sheets may harbor globally significant reservoirs of organic matter and be methane sources to the atmosphere (Wadham et al, 2008). Wadham et al. (2012) estimated a methane reservoir of ~10 Pg C beneath the WAIS, where thick organic rich sediments combined with low temperature and high pressure would promote methane hydrate storage. Methanogenesis has been reported in a number of subglacial environments (Boyd et al, 2010; Stibal et al, 2012a; Stibal et al, 2012b), including the bed of the Greenland Ice Sheet (Christner et al, 2012; Dieser et al, 2014). In the sediment core analyzed for this study, OTUs related to methanogenic archaea were rare (0.1%) and only detected in the lowest sediment depth analyzed (34-36cm). This coupled with measurements of hydrogenotrophic methane in sediment pore water suggests that methanogenic populations and activity may have been more prevalent in deeper portions of SLW's sediments (Michaud et al, submitted; Figure 2.4). An OTU (000112) closely related to the type I methanotroph, *Methylobacter tundripaludum* (99% identity), was ~10-fold lower in the water column than in the 0-2 cm sediment rRNA where it represented ~15% of the sequences (Figure 2.2). Dieser et al. (2014) identified a similar phylotype of *Methylobacter* that was inferred to be responsible for methane oxidation (320 nM CH_4 d^{-1}) in subglacial water outflows at the western margin of the Greenland Ice Sheet. The presence of active methanotrophy in SLW was supported not only by the high abundances of methanotroph-related OTUs and their large rRNA:rDNA ratios (Figure 2.3C), but also by geochemical and isotopic data which

showed a significant decrease in methane concentration between the surficial sediments (0-2cm) and the water column concurrent with a positive shift in the $\delta^{13}C\text{-}CH^4$ (Michaud et al, submitted). Together, these results indicate that microbial methane oxidation may serve a role as a substantial methane sink beneath the WAIS. The effect of subglacial methane release to the atmosphere during ice sheet wastage is estimated to be significant (e.g., Wadham et al, 2012), but such efforts have not considered aerobic methane oxidation as an aspect of carbon cycling beneath ice masses. Our data and those of Dieser et al. (2014) imply that bacterial methane consumption could be a significant methane sink and pathway for primary production in portions of the subglacial hydrological system where methane and oxygen coexist. Additional observations from polar subglacial environments are needed to provide the necessary parameterization required to model the biogeochemical contributions of the world's ice sheets to global carbon budgets.

Conclusion

There is a lack of fundamental information on the microbial biomes at the base of polar ice sheets because accessing these subglacial environments in a clean, environmentally-conscious way is technically and logistically challenging. The WISSARD project at SLW provided the first opportunity to examine the limnology and ecology of an Antarctic subglacial lake, revealing a chemosynthesis-based community comprised of bacteria and archaea, with no conclusive evidence for the presence of eukaryotic species (Christner et al, 2014; Achberger, unpub. data). The members of SLW's microbial community appear to derive their nitrogen, iron, sulfur, and carbon compounds from mineral weathering and relict organic matter in the sediments (Figure 2.4; Michaud et al, 2016), but various nutrients may be actively cycled in SLW and the interconnected WIS subglacial hydrologic system. At present it is unclear how representative the SLW microbial ecosystem is of other lakes in this region or to the many hundreds of others beneath the Antarctic ice sheet. However, taxa phylogenetically related to many of the dominant OTUs present in SLW have been previously observed in other icy subsurface

environments, raising possibilities for microbial biogeographical studies of specific taxa endemic to the polar regions. Most species inhabiting the SLW ecosystem were distinct from those entrapped in the overlying glacial ice (i.e., the water melted for drilling), implying that microbial inocula for the lake is derived from another source. Hence, microorganisms may enter into SLW as plankton or attached to particles that are transported by water. Alternatively, the contemporary inhabitants of SLW may represent ancient vestiges of marine communities that have persisted since past seawater intrusions to this region of West Antarctica.

CHAPTER 3.
METAGENOMIC ANALYSIS OF THE SUBGLACIAL LAKE WHILLANS MICROBIOME

Introduction

A vast liquid water reservoir exists beneath the Antarctic ice sheet in the form of extensive water saturated sediments (Priscu et al, 2008) and approximately 400 subglacial lakes (Smith et al, 2009; Wright and Siegert, 2012), a quarter of which are known to be hydrologically connected (e.g., Fricker et al, 2007). The logistical challenges associated with accessing such remote subsurface polar environments (Doran et al, 2008; Siegert et al, 2012) have limited the number of direct observations of conditions beneath continental ice sheets. The exploration of subglacial Lake Whillans (SLW), West Antarctica, in January of 2013 revealed that the ecosystem harbored diverse and active microbial communities (Christner et al, 2014). While much of what is known about subglacial ecosystem processes is based on physiological inferences using 16S rRNA and limited functional gene surveys, these data have suggested that communities may be sustained through chemosynthesis (e.g., Dieser et al., 2014; Hamilton et al, 2013; Achberger et al, submitted). Nonetheless, a comprehensive understanding of the functional potential of the microbial communities beneath the interior reaches of ice sheets is lacking.

The potential for active carbon fixation to occur in subglacial environments is supported by the detection of ribulose-1,5-bisphosphate carboxylase (RuBisCo) transcripts in subglacial water outflows from mountain glaciers (Boyd et al, 2014), the gene content of microbial assemblages preserved in the accreted ice of Subglacial Lake Vostok (Rogers et al, 2013), and measurements of dark CO_2-fixation in the SLW water column (Christner et al. 2014). Furthermore, the abundance of taxa closely related to species of *Sideroxydans lithotrophicus* and *Thiobacillus dentrificans* within SLW imply sulfur and iron oxidation pathways play a significant role in supporting primary production pathways within the lake (Achberger et al, in draft). Such organisms have been identified in other polar and alpine subglacial

environments where the oxidation of iron and sulfur compounds has been inferred through geochemical and functional gene (e.g., *aprA*) analyses (Lanoi et al, 2009; Mickucki et al, 2009, Hamilton et al, 2013; Boyd et al, 2014). Subglacial sediments have also been found to contain genes for ammonia oxidation (*amoA*), nitrate reduction (*narG*), and nitrogen fixation (*nifH*) lending support to the presence of active subglacial nitrogen cycling (Boyd et al, 2011). Based on the abundance of taxa closely related to ammonia oxidizing species of Thaumarchaeota and *Nitrosospira* as well as the nitrite oxidizing *Candidatus* Nitrotoga sp., nitrification appears to be another important chemolithoautrotrophic process occurring in SLW (Achberger et al, in draft).

Organic carbon stored beneath ice masses can be mineralized by methanogenic archaea (Boyd et al, 2010), providing substrate for methane oxidation (Dieser et al, 2014). Aerobic methanotrophy has been proposed to be a significant source of new organic carbon production in SLW (Michaud et al, submitted) where the abundance and distribution of 16S rRNA sequences related to the genus *Methylobacter* has implicated these taxa as being responsible for the oxidation of methane sourced from the deeper, anoxic sediments (Achberger et al, submitted; Michaud et al, submitted). The presence of taxa in SLW that are phylogenetically closely related to known lithoautotrophs and methanotrophs supports the possibility that their activities are important to maintain energy flow in subglacial lake ecosystems. However, since most microorganisms cannot be cultured in the laboratory and physiological predictions based on 16S rRNA gene data are tenuous, genome-based analyses provide the best available approach to unearth the metabolic pathways and potential for nutrient cycling in subglacial microbial communities.

Here we present an analysis of metagenomic data collected from size fractionated lake water samples (>3 μm, 3 to 0.8 μm, and 0.8 to 0.2 μm) and three sediment depths (0-2 cm, 4-6 cm, and 14-16 cm) to provide detailed information on the composition and functional potential of microorganisms inhabiting SLW. The taxonomic classification of sequences revealed a dominance of bacterial and

archaeal taxa with only a minor eukaryotic component to the subglacial community. Genes involved in the oxidation of sulfide and ammonia as well as those for aerobic carbon fixation were also associated with abundant members of the SLW community (e.g., *Thiobacillus* and *Nitrosospira*), supporting the hypotheses that reduced nitrogen and sulfur compounds derived from the weathering of organic matter deposits and bedrock minerals support primary production within the SLW system.

Materials and Methods

Site Description and Sample Collection

SLW is located beneath ~800m of ice on the lower Whillans Ice Stream (WIS), West Antarctica, has a maximum area of ~60km^2, and is part of a subglacial hydrologic network connecting lacustrine features beneath the upper WIS and the neighboring Kamb Ice Stream (Fricker and Scambos, 2009; Christianson et al, 2012). Studies of subglacial water transport based on surface ice elevation changes indicate that SLW experiences episodic filling and draining cycles, with water exiting the lake and eventually draining into the Ross Sea at the WIS grounding zone (Fricker and Scambos, 2009; Pritchard et al, 2012; Wright and Siegert, 2012; Carter et al, 2013). Since 2003, SLW has filled and drained on three occasions, making it one of the more active lakes in the region (Siegfried et al, 2016). During January 2013, the Whillans Ice Stream Subglacial Access Research Drilling (WISSARD) project created a borehole in the ice overlying SLW (drill location 84.240^{o}S 153.694^{o}W) using a microbiologically clean hot water drill (Priscu et al, 2013; Blythe et al, 2014; Burnett et al, 2014; Rack et al, 2014), allowing sampling of the water column and lake sediments (Tulaczyk et al, 2014).

At the time of sampling, the water column of SLW was 2.2m deep and the lake was in the process of slowly filling following a drainage event in 2009 (Siegfried et al, 2016). The lake water, sourced primarily from glacial melt, and the upper ~15cm of underlying sediments were inferred to be oxic (Christner et al, 2014; Michaud et al, 2016). The sediment pore waters were found to have a minor

seawater solute component that increased with depth (Michaud et al, 2016). This marine signature has been attributed to the presence of buried sediments deposited during the Pleistocene when the area was inundated with ocean waters (Scherer et al, 1998; Michaud et al, 2016). Details on the physical and chemical properties of SLW are provided in Christner et al (2014), Michaud et al (2016), and Vick-Majors et al (submitted).

Samples of suspended particulates in the SLW water column were collected by filter concentrating 5.3L of water (i.e., the second of three casts; Achberger et al, submitted) sequentially through a 3.0, 0.8, and 0.2 µm filter membrane *in situ* with a large volume water transfer system (WTS-LV; McLane Research Laboratories Inc.). The recovered filters were sectioned and preserved as described by Christner et al. (2014). A shallow sediment core was recovered using a Uwitec multicoring device and frozen immediately upon retrieval (-20˚C). Once back in the United States, the sediment core was thawed at 4˚C and subsampled as described previously in Achberger et al. (submitted). Samples collected for nucleic acids were then refrozen until extraction.

DNA Extraction and Sequencing

DNA extraction from filters was conducted as previously described in Achberger et al, (in draft). Extraction of DNA from SLW sediments followed the protocol of Lever et al (2015). Briefly, 0.5g wet weight sediment was added to a tube containing sterile 0.1mm zirconia/silica beads and a solution of deoxynucleoside triphophastes (dNTPs; final concentration of 450 µmoles PO_4^{2-}) to minimize adsorption of extracted DNA onto clay-rich sediments. Lysis buffer I (1.5 mL; Lever et al, 2015) was then added to the sediment slurry and microbial cells were physically lysed with two freeze-thaw cycles at -80°C and a 1 h incubation at 50°C followed by 30 s of bead beating at high speed in a tissue homogenizer (Qiagen). Extracted DNA was then purified with a chloroform-isoamylalcohol solution and precipitated using 100% ethanol, 5M NaCl, and 20 µL mL^{-1} (final concentration) linear polyacrylamide at room temperature for 2

h. Precipitated DNA was centrifuged for 45 min at room temperature, resuspended in DNAse/RNAse-free water, and further cleaned with the Norgen Clean All RNA/DNA kit (Norgen Biotek).

The DNA was quantified fluorometrically using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies). To generate metagenomes from water samples, 1.2, 2.9, and 2.8 ng of DNA were used for the 3, 0.8, and 0.2 μm filters, respectively. For the sediment metagenomes, 0.15, 1.3, and 2.6 ng of DNA were used for the 0-2, 4-6, and 14-16 cm samples, respectively. The Illumina Hi-Seq platform was used to generate 100 bp paired end reads from DNA extracted from the water and sediment samples, and was performed by the Oregon State University Center for Genome Research and Biocomputing and Marine Biological Laboratory, respectively.

Metagenomic Sequence Analysis

The ~100 million paired end reads generated from metagenomic sequencing were screened for quality, filtered, and trimmed using Trimmomatic (v0.32; Bolger et al, 2014). The unassembled reads were uploaded to MG-RAST (MetaGenome Rapid Annotation with Subsystem Technology; Meyer et al, 2008) and predicted genes were annotated with KEGG (Kyoto Encyclopedia of Genes and Genomes; Kanehisa and Goto, 2000). The normalized abundance of genes associated with KEGG pathways was compared between samples using Welsh's t-test and Benjamini-Hochberg FDR multiple test corrections, performed in STAMP (Statistical Analysis of Metagenomic Profiles; Parks et al, 2014). Reads assigned to KEGG orthologs (KO) that represented marker genes involved in carbon, nitrogen, and sulfur cycling (Supplemental Table B.1) were identified and analyzed as described by Lauro et al, (2011). Heat plots were generated in R (v.3.1.2; RC Team, 2015).

DNA sequences from each sample were assembled using IDBA-UD (Iterative De Bruijn graph Assembler for uneven depth; Peng et al, 2012). Assembly statistics are shown in supplemental Table B.2. Assembled contigs larger than 500bp were annotated with IMG (Integrated Microbial Genomes;

Markowitz et al, 2012) and those taxonomically affiliated with organisms of interest (e.g., numerically abundant based on 16S rRNA data; Achberger et al, in draft) were examined in detail to further evaluate their potential ecological role to the SLW system.

Results

Microbial Community Structure of Subglacial Lake Whillans

The majority of the metagenomic sequences obtained from samples of SLW's sediment (0-2cm, 4-6cm, and 14-16cm) and water column were taxonomically affiliated with bacteria (≥87%, Table 3.1). The abundance of archaeal sequences was greater in the water column compared to the sediment data, with the 0.2 µm filter size fraction being the highest (Table 3.1) and ~98% of the archaeal sequences classifying as Thaumarchaeota (Figure 3.1). Similarly, archaeal species richness also appeared limited in the sediment profile, with many of the sequences related to those of methanogenic Euryarchaeota (e.g., *Methanosarcinales* and *Methanomicrobiales*). No more than 0.6% of the sequences from the libraries were assigned to eukaryotic and viral taxa (Table 3.1). The most abundant Eukaryotic phylum identified was that of the Cnidaria with reads predominantly found on the 3.0 um filter and most closely related to species of *Hydra*.

Table 3.1. The relative abundance of metagenomic reads for the SLW water column and sediments.

	Water			Sediment		
	3.0um	**0.8um**	**0.2um**	**0-2cm**	**4-6cm**	**14-16cm**
Archaea	1.3%	4.9%	12.0%	0.6%	0.5%	1.2%
Bacteria	97.5%	94.4%	87.3%	98.2%	98.6%	97.5%
Eukarya	0.3%	0.1%	0.1%	0.2%	0.1%	0.2%
Viruses	0.1%	0.1%	0.1%	0.4%	0.3%	0.4%
other	0.8%	0.5%	0.5%	0.7%	0.4%	0.7%

Analysis of the bacterial sequence reads indicated that members of the Betaproteobacteria were most abundant (32-57%; Figure 3.1). Within the SLW water column, sequences mapping to the

genomes of *Polaromonas*, *Sideroxydans*, and *Gallionella* species were prevalent, and together represented 16 to 26% of classifiable reads from the filter size fractions (Figure 3.2). These genera were also inferred to be abundant in the surficial (0-2cm) sediments which contained numerous sequences related to *Thiobacillus* (~8%; Figure 3.2). The community composition changed substantially with depth, and the sediment data from 4-6 cm horizon was largely comprised of Actinobacterial sequence reads affiliated with the genus *Rhodococcus* (Figures 3.1 and 3.2). Also abundant in the 4-6 cm sediment data were reads associated with the genera *Agrobacterium*, *Achromobacter*, and *Bordetella* (Figure 3.2). Overall, the taxonomic composition of communities at sediment depths of 4-6 cm and 14-16 cm was similar (Figures 3.1 and 3.2).

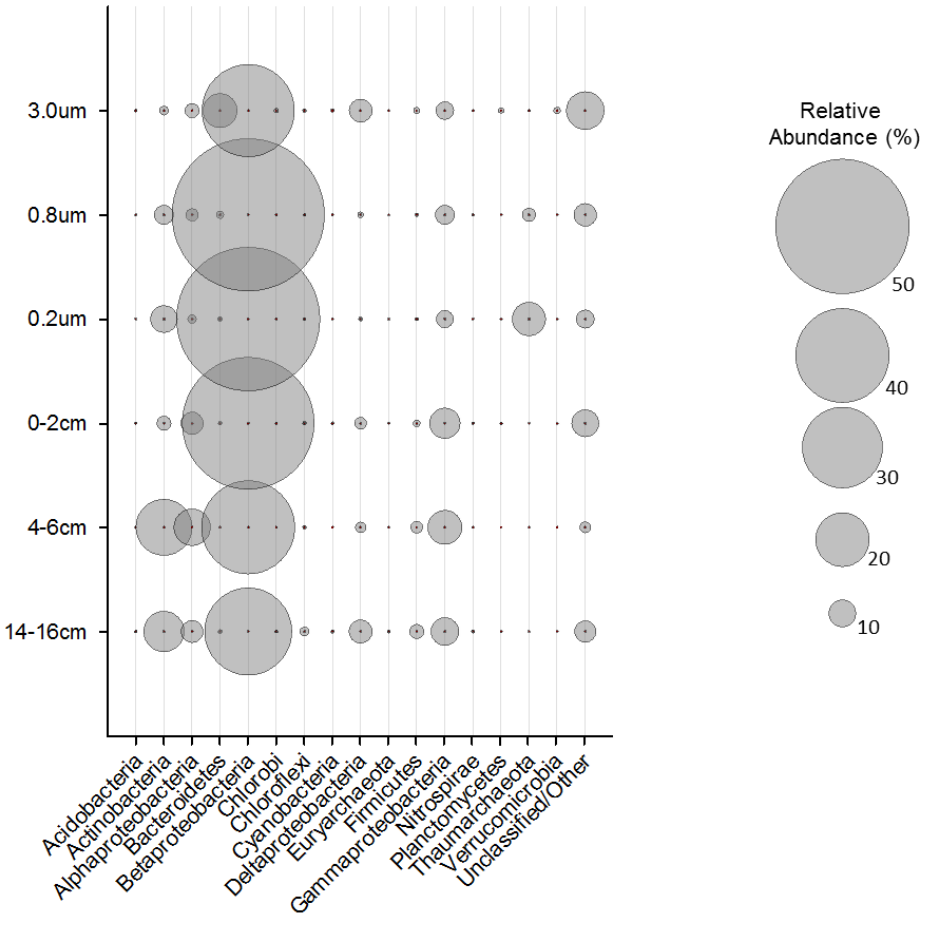


Figure 3.1. Phylum level classification of metagenomic reads from SLW. All groups that represented <1% of reads are condensed into the Unclassified/Other category.

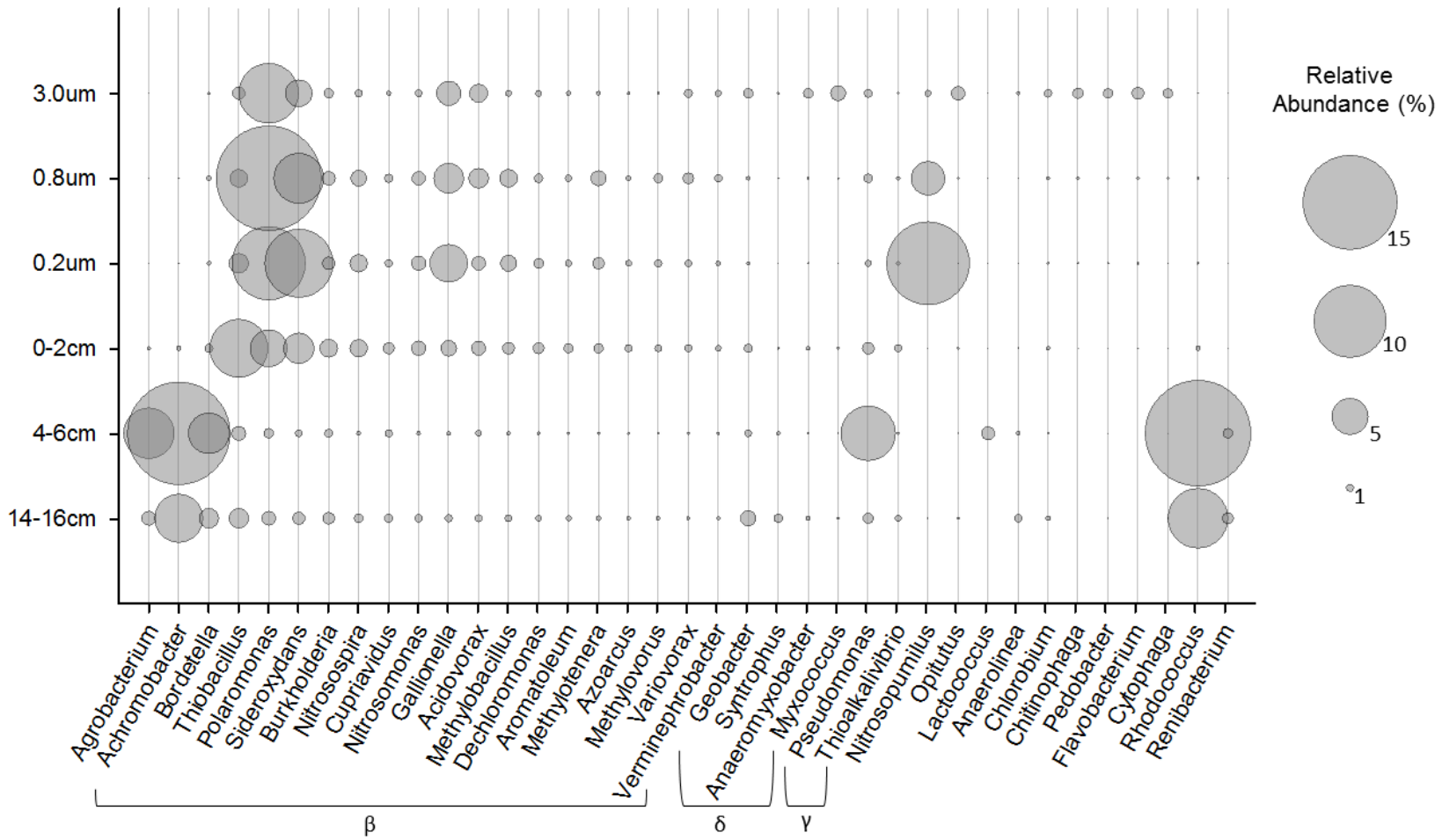


Figure 3.2. The most abundant genera in SLW water column and sediments metagenomic datasets. Only those groups that represented at least 1% of the reads in a given sample are listed.

Functional Potential of the Subglacial Lake Whillans Microbial Community

Metagenomic reads assigned to KEGG pathways were used to assess differences in the functional composition between distinct habitats in SLW (Figure 3.3). Genes associated with transcription, translation, replication, repair, and metabolism of amino acids and nucleotides were all statistically more abundant in the water column compared to the sediments (Figure 3.4A). One of the most notable difference between the two environments was seen in the category of membrane transport (Figure 3.4A) where genes associated with ABC transporters were ~2 to 9% more abundant in the sediment profile than in the water column (Supplemental Figure B.1A). Of those, genes associated with the transport of peptides, dipeptides, and branched-chain amino acids had the highest relative abundance in the sediments (Supplemental Figure B.1B) and many were taxonomically related to species of *Achromobacter* (22% of genes).

The overall genetic potential for the metabolism of carbohydrates was similar throughout SLW (Figure 3.4C) however, distinct differences were seen among the constituent pathways (Figure 3.4C). Genes associated with glycolysis were the most highly represented in all samples and statistically more abundant in the water column than in the sediments, while the sediments contained a greater number of genes involved in the metabolism of butyrate, propionate, and galactose (Figure 3.4C). Variability could be seen between depths in the sediment profile. For example, genes involved in the metabolism of glyoxylate were most numerous in the 0-2cm horizon and genes associated with starch/sucrose metabolism showed an increased abundance with depth (Figure 3.4C). The distribution of marker genes for fermentation also suggests that this process occurs in the deepest portions of the sediment core sampled (14-16cm; Figure 3.3) and most likely results in the production of CO_2 and ethanol (data not shown).

Markers for methanogenesis were only found in the metagenomic data generated from the sediments and were most prevalent in 4-6cm and 14-16cm horizons where they were taxonomically

affiliated with members of the Methanomicrobia (100%; Figure 3.3). Despite the presence of metagenomic reads related to methanotrophic organisms (e.g., *Methylobacillus* and *Methylovorus*; Figure 3.2) markers for methane oxidation affiliated with these groups were not found. Overall, genes associated with methane metabolism were higher in the sediments than the overlying water column (Figure 3.4B).

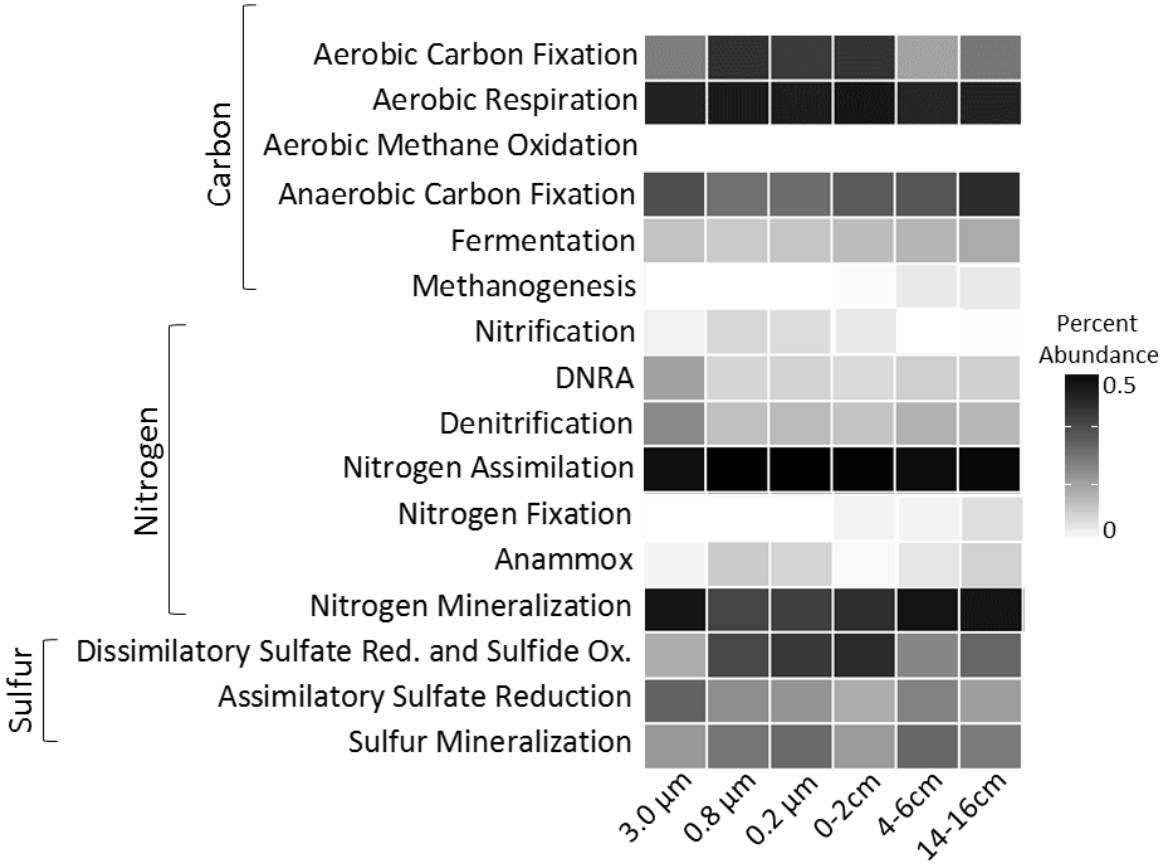


Figure 3.3. Heat plot showing the abundance of marker genes for carbon, nitrogen, and sulfur metabolic pathways in the SLW water column and sediment. Abundances were calculated based on normalized number of total annotated reads for each sample.

Pathways for carbon fixation were most highly represented in metagenomic data from the water column (Figure 3.4B) where aerobic carbon fixation via the Calvin-Benson-Bassham (CBB) cycle was most prevalent (Figure 3.3). Of the marker genes for the CBB cycle found in the lake water and surficial sediments (0-2cm), ~28% of reads were affiliated with species of *Thiobacillus*, *Nitrosospira,*

Gallionella, and *Sideroxydans.* This is in agreement with the phylogenetic analysis of assembled RuBisCo gene fragments which showed that the majority were related to Form IA/C and Form II, often associated with obligate and facultative chemolithotrophs (Supplemental Figure B.3). Anaerobic carbon fixation pathways were most prevalent at sediment depths of 14-16cm (Figure 3.3) where marker genes associated with the reductive tricarboxylic acid (rTCA) cycle were most abundant and taxonomically related to Deltaproteobacteria (e.g., Desulfobacterales and Syntrophobacterales). Reads mapping to marker genes for other carbon fixation pathways such as the reductive acetyl-CoA pathway and the 3-hydroxypropionate/4-hydroxybutryrate cycle were rare in the metagenomic datasets.

Marker genes involved in nitrogen assimilation were abundant in all samples while those associated with mineralization were greatest in the 4-6cm sediment horizon (Figure 3.3) and largely affiliated with species of *Rhodococcus* (19%). The potential for denitrification and dissimilatory nitrate reduction to ammonia (DNRA) was low in the SLW samples (Figure 3.3), and genes associated with these processes were most similar to those in species of *Cupriavidus* (~16%) and *Anaeromyxobacter* (~30%), respectively. Overall, markers for nitrogen fixation and anaerobic ammonia oxidation (anammox) were infrequent in the data (Figure 3.3). Genes involved in nitrification (specifically ammonia oxidation) were related to species of *Nitrosomonas* and *Nitrosospira* (50-75% combined). However, metagenomic reads related to these ammonia oxidizing bacterial species were two-fold less abundant in the water column than those related to *Nitrosopumilus*, an ammonia oxidizing Thaumarchaeota (Figure 3.2). Several of the metagenomic assemblies contained genes that are phylogenetically related to species of Thaumarchaeota, including genes encoding ammonia monooxygenase enzyme subunits (e.g., *amoC)* and those associated with carbon fixation via the 3-hydroxypropionate/4-hydroxybutryrate pathway. Genes involved in urea transport and utilization (*ureABC* and *ureEFGH*) were also found on large genomic fragments together with 16S and 23S rRNA genes (Supplemental Figure B.2) that were most

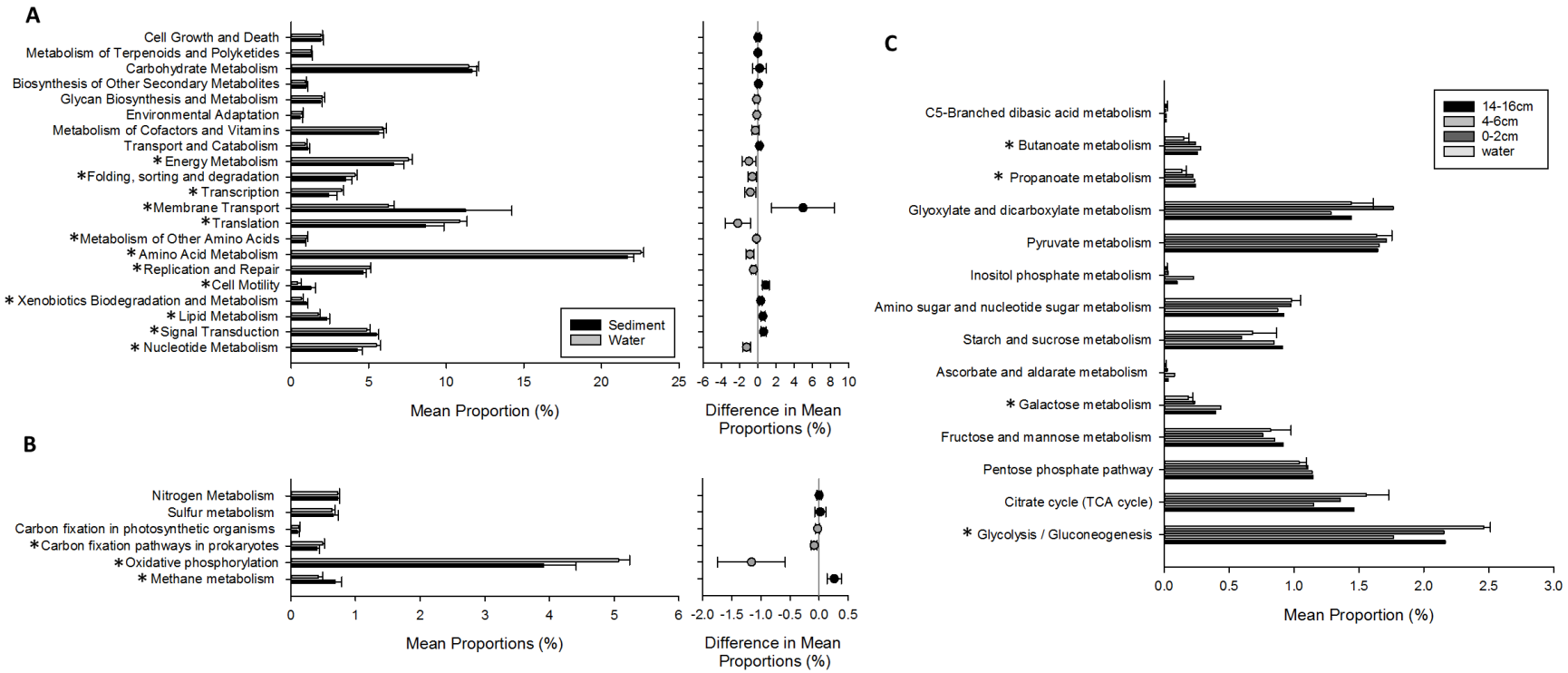


Figure 3.4. The abundance of (A) hierarchal KEGG pathways, (B) energy generating metabolic processes, and (C) carbohydrate metabolic pathways for the SLW water column and sediment microbial community. Asterisks indicate statistically different groups.

closely related to *Candidatus* Nitrosopumilus koreensis AR1 (97% Identity) and shared 100% 16S rRNA gene identity (V4 region) to the dominant archaeal OTU detected in 16S rRNA gene surveys of the water column (Achberger et al, submitted).

The genomic potential for microbially driven sulfur cycling was also present in the SLW communities (Figure 3.3). Reads mapping to markers for assimilatory sulfate reduction were most numerous in the 3.0µm water column sample and at a depth of 4-6cm within the sediment profile (Figure 3.3) where they were taxonomically related to *Gallionella* (10%) and *Rhodococcus* (26%), respectively. The potential for sulfur mineralization was also highest in the water column (0.8 and 0.2µm) and in the 4-6cm sediment horizon (Figure 3.3) and was associated with species of *Polaromonas* (20%) and *Rhodococcus* (30%), respectively. Genes involved in dissimilatory sulfate reduction and/or sulfide oxidation pathways (i.e., dissimilatory sulfite reductase, *dsrA*; adenylylsulfate reductase, *aprA*) were most abundant in the smallest size fractions from the water column (0.8 and 0.2 µm) and in the surficial sediments (Figures 3.3), and related to *Thiobacillus* species (34-42%). Genes encoding the SOX enzyme complex (i.e., *soxXYZABCD*) were not found in the examination of raw reads however, they were identified in assembled metagenomic sequences where they were found throughout the sediment profile but were absent from the water column. Within the surface sediments (0-2cm), SOX genes (*soxXYZABCD*) were also most closely related to species of *Thiobacillus*, while genes at 4-6cm and 14-16cm were associated with species of *Achromobacter* and *Sulfuricella,* respectively.

Discussion

Due to its remoteness and concealment beneath kilometers of ice, the vast majority of environments on the Antarctic continent are understudied. Based on the few existing data, subglacial aquatic environments beneath the Antarctic ice sheet are estimated to harbor ~10^{29} prokaryotic cells (Priscu et al, 2008); a pool equivalent to that found globally in open ocean waters (Whitman et al, 1998).

An improved understanding of the microbiota and metabolic processes occurring beneath the world's ice sheets is necessary because their role in global biogeochemical cycles remains unknown. Large amounts of subglacial runoff (~53 km^3y^{-1}) produced by basal melting drain from the Antarctic continent into the Southern Ocean, transporting nutrients and biogenic greenhouse gases (e.g., CH_4) that may affect marine microbial productivity and current models of biogeochemical cycling (Wadham et al, 2013; Michaud, 2016; Vick-Majors, 2016). The recent exploration and recovery of samples from SLW has provided the first opportunity to test hypotheses on microbial ecosystem function in an Antarctic subglacial lake and better understand their role in biogeochemical reactions that control fluxes of organic carbon, nutrients, and climate-relevant gases.

The community structure of SLW as determined by analysis of metagenomic data was found to be dominated by bacterial and archaeal species with only a minor eukaryotic and viral component. The scarcity of eukaryotic reads identified through metagenomic sequencing is consistent with prior attempts to amplify 18S rRNA genes from samples which were unsuccessful (Christner et al, 2014). In accretion ice from Subglacial Lake Vostok, East Antarctica, and sediments for Robertson Glacier, Canada, eukaryotic taxa were also infrequent with species of fungi, ciliates, and amoeba identified most often (Hamilton et al, 2013; Rogers et al, 2013). Although sequences phylogenetically related to such organisms were detected in the metagenome data, they were rare with the most abundant taxa related to *Hydra* within the Cnidaria. Sequences related to this genus were predominantly found on the 3.0 μm filter as expected based on their reported size range (>2mm long; e.g., Slobodkin et al, 1991) however, they could also be detected in all other samples collected from SLW. Consequently, it is unclear if the detection of such organisms is the result of possible contamination during sample handling and sequencing or if the sequences represent organisms that are currently active or preserved (i.e., relic DNA) within the environment. Additionally, the relative abundance of viruses in SLW as estimated through metagenomic sequencing (Table 3.1) is likely an underrepresentation of the true viral load

within the subglacial environment given that the method for water sampling excluded all particles <0.2um and that the nucleic acid extraction methods were not optimized or tested for viral genomic DNA recovery.

Many of the dominant bacterial and archaeal taxa (e.g., *Polaromonas*, *Thiobacillus*, Thaumarchaeota, and *Sideroxydans*) observed in the metagenomic datasets from the SLW water column and surficial sediments (0-2cm; Figure 3.2) were the same as those previously identified through the 16S rRNA gene based survey (Achberger et al, submitted). One notable difference was the lack of metagenomic reads classifying to *Candidatus* Nitrotoga arctica (Alawi et al, 2007), the closest relative of several abundant OTUs within the lake (Achberger et al, submitted). Given the limited genomic information available on this species, it is possible that sequences originating from such organisms were taxonomically assigned to other closely related species such as those of *Gallionella* and *Sideroxydans* (e.g., Alawi et al, 2007). Within the deeper sediments (4-6cm and 14-16cm) metagenomic sequences affiliated with *Agrobacterium*, *Achromobacter*, and *Rhodococcus* were most abundant (Figure 3.2). These genera were either absent or very rare (<0.001%) in the 16S rRNA gene libraries. However, in general, many of the dominant OTUs found within the sediment profile were poorly classified (<90% identity; Achberger et al, submitted) and therefore it is possible that more abundant members of these genera were overlooked.

Microbial communities beneath ice sheets are isolated from sunlight and direct input from the surface, and are therefore dependent on metabolic substrates and nutrients released from melting basal ice or products generated from biotic and abiotic weathering reactions (Skidmore 2011; Mitchell et al, 2013). The water in SLW is predominately derived from glacial melt (Christner et al, 2014), and while this glacial water provides biologically important gasses such as O_2, CO_2, and N_2, it contains relatively low concentrations of organic matter and other nutrients (e.g., Christner et al. 2006). However, sediment porewater residency time estimates (1 to 10 kyr; Christoffersen et al, 2014) suggest

ample timeframes for weathering processes to occur and alter the composition of the SLW water column (Michaud et al, 2016). Due to its close proximity to the grounding zone (~100 km), past marine intrusion in the area (e.g., Scherer et al, 1998) has historically impacted the modern day site of SLW. Evidence for this marine influence was found in the solute and isotopic composition of the lake at the time of sampling and revealed that the seawater component increased from ~2.6% in the water column to ~6% in the deepest sediments sampled (40cm; Michaud et al, 2016). Much of the WAIS is thought to be underlain with marine sedimentary deposits, containing an estimated 6000 Pg C (Wadham et al, 2012). This ancient pool of organic matter may serve as substrate for microbial metabolism and consequently provide additional nutrients (e.g., ammonia and methane) to fuel ecosystem processes (Bardgett et al, 2007; Brankatschk et al, 2011; Wadham et al, 2012; Bradley et al, 2014; Christner et al, 2014).

SLW contained a pool of dissolved organic carbon (DOC) in excess of that needed to sustain the estimated heterotrophic demand in the water column (Christner et al, 2014; Vick-Majors, 2016). Based on measured rates of dark ^{14}C incorporation (Christner et al, 2014), it unlikely that the DOC pool could have been solely derived from *in situ* microbial primary production, and may therefore be sourced in part from the sediments which showed an increasing concentration of DOC with depth (Vick-Majors, 2016). Characterization of the dissolved organic matter (DOM) of SLW using excitation-emission matrix spectroscopy revealed that within the water column and surficial sediment pore waters, the DOM was predominantly amino acid-like (tryptophan and tyrosine), indicative of microbial production (Vick-Majors, 2016). This amino acid-like signature gradually decreased with depth, coinciding with an increase in humic-like DOM which may represent a more recalcitrant pool of organic matter (Vick-Majors, 2016). Species of *Polaromonas* and *Rhodococcu*s, two of the most abundant organisms in the lake water and sediments (Figure 3.2) are known for their ability to metabolize a variety of complex organic molecules (Malachowsky et al, 1994; Mattes et al, 2008; Smith et al, 2014). Indeed, an

evaluation of assembled genomic fragments associated with these genera revealed that they have numerous genes associated with the degradation of small aromatic compounds (e.g., phthalate, toluene, xylene) and may therefore be able to metabolize organic compounds inaccessible to other organisms in SLW. The prevalence of genes involved in the transport of branched-chain amino acids and peptides also suggests that proteinaceous compounds (Supplemental Figure B.1) may be an important carbon source for the sediment microbial community.

The abundance of genes associated with several pathways for carbohydrate metabolism differed significantly between the microbial communities within the sediment profile and water column (Figure 3.4C). Among such pathways were those for the metabolism of butyrate, propionate, and glyoxylate (a potential pathway for acetate assimilation; e.g., Ensign et al, 2006; Figure 3.4C). These compounds can be generated through fermentative processes which are likely to occur primarily in the sediments (Figure 3.3). Within the deepest sediments sampled (14-16cm), CO_2 is predicted to be one of the major fermentation products, and may in turn serve as a carbon source for chemolithoautotrophic species which are inferred to be abundant in SLW (Achberger et al, submitted).

The relic marine carbon beneath the Antarctic ice sheets is hypothesized to fuel acetoclastic methanogenesis and consequently, the generation of a globally significant pool of methane (Wadham et al, 2012). Samples collected from SLW provided the first opportunity to test such hypotheses. The deuterium and carbon isotope values for the dissolved methane in the SLW sediments (75-300 μM CH_4) indicate that it was produced via hydrogenotrophic methanogenesis rather than through acetoclastic or thermogenic processes (Michaud et al, in draft). The hydrogen needed to fuel methanogensis in SLW may be supplied from a number of sources including fermentation, glacial comminution of bedrock, and/or hydrothermal sources (e.g., Telling et al, 2015; Skidmore et al, 2011).

Genes associated with methanogenesis (e.g., *mcrAB*) were rare within SLW and only found in

the deeper sediments (4-16cm; Figure 3.3). However, previous analysis of 16S rRNA derived datasets did not detect potentially active methanogenic taxa in the upper 20 cm of the SLW sediment profile and showed that they were rare (<0.1%) in the 34-36cm horizon (Achberger et al, submitted). These observations, combined with the inferred redox profile in the sediments, suggest that methane production may have predominantly occurred in deeper sediment horizons (>40cm; Michaud, 2016).

Methane consumption by aerobic methanotrophs inferred to be abundant in the surficial sediments could represent a significant pathway of primary production in SLW (Achberger et al, submitted). In light of this, the absence of marker genes (e.g., *pmoA*) for aerobic methane oxidation (Figure 3.3) was unexpected. However, sequences of the *pmoA* gene were amplified from SLW sediments (>16cm) that were related to *Methylobacter tundrapaludum*, the dominant methanotrophic taxa identified in 16S rRNA gene surveys (Michaud et al, in draft; Achberger et al, submitted). This species was also inferred to play a significant role in the oxidation of methane in Greenland subglacial environments (Dieser et al, 2014). A comparison of the *pmoA* sequences recovered from SLW and Greenland revealed that they shared 95% identity, which based on an OTU cutoff of >93% (e.g., Degelmann et al, 2010), suggests that the two methanotrophic taxa in these geographical distal locations are highly related and may be well suited for life in cold subglacial environments.

Based on the rate of dark CO_2 fixation (Christner et al, 2014), lithoautotrophic primary production was inferred to be the dominant source of organic carbon compounds in the SLW water column (Vick-Majors, 2016). Consistent with this finding, genes for aerobic carbon fixation (i.e., CBB cycle) were abundant in the water column and sediment metagenomes (Figure 3.3), and taxonomically associated with species of *Thiobacillus*, *Nitrosopira*, *Sideroxydans*, and *Gallionella* (Figure 3.2; Supplemental Figure B.3). These results agreed with community profiles based on 16S rRNA data that showed species of *Thiobacillus*, *Nitrosopira*, and *Sideroxydans* were abundant members in the SLW ecosystem (Achberger et al, submitted). The gene marker for the 3-hydroxypropionate/4-

hydroxybutryrate cycle (Supplemental Table B.1) used for carbon fixation by members of the Thaumarchaeaota was not detected in the analysis of unassembled reads but it and many of the other genes involved in this pathway were identified on assembled genomic fragments associated with this group. The Thaumarchaeota are among the most abundant members of the SLW water column (Figures 3.2 and 3.3) and may therefore play an important role in primary production within the lake. Rates for anaerobic carbon fixation were not determined for SLW however, genes associated with these pathways (e.g., rTCA and reductive acetyl CoA) were found in the deeper sediments (14-16cm; Figure 3.3) where they were mainly associated with sulfate-reducing organisms.

The nutrient composition of the SLW water column implied it was biologically deficient in nitrogen compared to carbon and phosphorous (Christner et al, 2014). Ammonium was the primary inorganic nitrogen species in the lake (Christner et al, 2014) and based on enriched ammonia concentrations in sediment porewaters, was likely supplied to the water column by diffusion (Vick-Majors, 2016). The genetic potential for nitrogen fixation in SLW was low (Figure 3.3), and although mica and feldspar weathering reactions could produce small quantities of dissolved nitrogen species (Tranter and Wadham, 2014), the main source of ammonia is likely from the microbial mineralization of organic matter stored in the subglacial sediments (Figure 3.3). Marker genes for anammox were poorly represented among the metagenomic data (Figures 3.3) supporting previous 16S rRNA analyses from SLW which showed a low abundance of active taxa capable of this process (Achberger et al, submitted). Genes involved in the aerobic oxidation of ammonia were primarily found within the water column (Figure 3.3) and associated with various ammonia oxidizing bacterial (AOB) taxa (e.g., *Nitrosospira* and *Nitrosomonas*). This conflicted with the taxonomic composition of known ammonia oxidizing species inferred from the metagenome (Figure 3.2) and 16S rRNA data, which suggested that archaeal ammonia oxidizers (AOA) dominated the water column, while AOB were more prevalent in the lake sediments (Achberger et al, submitted). Multiple *amo* gene operons (2-3 copies) are generally present in the

genomes of AOB (Norton et al, 2002) compared to known AOA which have only a single copy (Hatzenpichler, 2012). Perhaps because of this, the contribution of AOB to water column nitrification may be overestimated based on the metegenomic analysis. Assembled genomic fragments associated with the AOA in SLW were found to contain genes involved in ammonia oxidation (e.g., *amoBC*) and interestingly also had genes need for the metabolism of urea (Supplemental Figure B.2). Urea is proposed to be an important energy and carbon source for species of Thaumarchaeota inhabiting Arctic and Antarctic polar waters (Alonso-Saez et al, 2012; Connelly et al, 2014). Although the concentration of urea in SLW was not determined, if present, it may also serve to fuel nitrification within the lake.

The genes involved in denitrification and DNRA were highest in the deeper sediments and on the 3.0µm filter fraction, resembling the distribution of markers for anaerobic carbon fixation (Figure 3.3). Given that the SLW water column was aerobic at the time of sampling, the genomic potential for such processes to occur in the lake may suggest the presence of anaerobic microenvironments within suspended sediment particles, similar to what has been found in marine snow aggregates (e.g., Stief et al, 2016). The water of SLW was heavily laden with particulates (Tulaczyk et al, 2014), providing ample substrate for microbial attachment and metabolism.

Genes associate with dissimilatory sulfate reduction and sulfide oxidation were most abundant in the aerobic water column and surficial sediments (Figure 3.3). Although the enzymes encoded by the marker genes (e.g., *aprA* and *dsrA*) for these pathways function in both oxidative and reductive processes, they were predominantly related to sulfide oxidizing bacteria (SOB) such as *Sideroxydans* and *Thiobacillus.* This was consistent with previous findings showing that 16S rRNA, *aprA,* and *dsrA* genes from these taxa were abundant in SLW sediments (Purcell et al 2014; Achberger et al, submitted). Members of these genera have also been detected in sediment samples from alpine glaciers (Boyd et al, 2014) and beneath the Kamb Ice Stream, West Antarctica (Lanoil et al, 2009), suggesting they are important members in a number of subglacial ecosystems where the oxidation of sulfide minerals is

thought to be a dominant metabolic process (e.g., Skidmore et al, 2011). The number of metabolic and 16S rRNA genes related to sulfate reducing taxa was low (Achberger et al, in draft), supporting chemical profiles of sulfate in the sediment porewaters (Michaud et al, 2016) and suggesting that sulfate reduction was not an active microbial process at the time of sampling.

Conclusion

The exploration of SLW has provided an unprecedented opportunity to evaluate the suitability of Antarctic subglacial lakes as habitats for microorganisms and understand the energy sources that drive ecosystem processes. Based on molecular (Achberger et al., submitted; this manuscript) and biogeochemical (Christner et al. 2014; Majors-Vick, 2016, Michaud et al, 2016) data, primary production pathways are dependent on the oxidation of reduced sulfur compounds, ammonia or urea, and methane. The principal sources of substrate for these reactions are likely a combination of abiotic and biotic processes involved in the weathering of minerals at the bed and decomposition of organic matter reservoirs in the underlying sediment. Several members of the SLW ecosystem (e.g., *Thiobacillus*, *Sideroxydans*, and *Methylobacter*) have also been detected and identified as important species in other subglacial environments, raising the possibility that these microorganisms may occupy the same ecological niches in geographically distal locations. Although eukaryotic sequences were detected in SLW, the potential for active populations to inhabit subglacial environments remains largely unknown. Additionally, the present study did not enable a comprehensive analysis into the diversity and ecological importance of viruses in the subglacial environment, which merits further investigation. Molecular surveys of SLW combined with physiological and geochemical data have provided a holistic model for how microbially mediated nutrient cycling may occur in aquatic environments beneath ice masses.

CHAPTER 4.
BACTERIAL AND ARCHAEAL DIVERSITY OF A DEEP SEA ECOSYSTEM IN PROXIMITY TO THE ROSS ICE SHELF GROUNDING ZONE

Introduction

Three-quarters of Antarctica's coast is lined with ice shelves; floating ice masses that serve as conduits for grounded ice transport into the Southern Ocean. Ice shelves are important for the stabilization of the Antarctic Ice Sheet (AIS) through the buttressing of continental ice and influence ice sheet dynamics (Goldberg et al, 2009). As a result, accelerated ice flow and significant ice mass loss has been attributed to ice shelf collapse (De Angelis and Skvarca 2003; Rignot et al, 2004). The grounding line, the transition point at which grounded ice masses become buoyant, is a critical region that affects ice shelf stability and migrates in response to changes in ice thickness and sea level (Goldberg et al, 2009). As such, the dynamics of grounding zones have been intensely studied due to their oceanographic and glaciological relevance, particularly in West Antarctica were the marine ice sheet is thought to be especially sensitive to grounding zone stability (Mercer, 1978; Thomas, 1979; De Angelis and Skvarca, 2003; Rignot et al, 2004; Goldberg et al, 2009; Brunt et al, 2010). The discovery of a subglacial hydrologic network beneath the AIS that transports large volumes of melt water from the continent's interior to the ocean (Smith et al, 2009; Wright and Siegert, 2012) have prompted investigations to examine the effect of subglacially derived water and sediment on the grounding zone environment (Carter and Fricker, 2012; Horgan et al, 2013). However, there are few direct observations of grounding zone conditions and the biological diversity of ecosystems poised at these important transitional sites have not been systematically investigated.

Although studies focusing on the macro- or micro-ecology at grounding zones are limited, several observations have provided information on the properties of sub-ice marine cavities beneath Antarctic ice shelves. Rich benthic communities were observed up to 200 km from the edge under the Amery Ice Shelf that were hypothesized to be supported by organic carbon and nutrients that advected

beneath the ice sheet from the open ocean (Riddle et al, 2007; Post et al, 2014). Additionally, sparse populations of fish and crustaceans were also reported at the J9 station on the Ross Ice Shelf (RIS; Lipps et al, 1979), which was more than 400 km from the shelf front. At this site, Horrigan (1981) proposed that nitrifying populations in the water column (Azam et al, 1979), could serve as primary producers, providing enough fixed organic carbon to the system to the support the observed macrofauna. Following the collapse of the Larson B Ice Shelf, studies found that the interior benthic communities resembled those of the deep sea but transitioned to continental shelf types near the former ice margin, suggesting changes in food availability (Rose et al, 2015).

Recent surveys of sub-ice shelf microbial diversity have been conducted on the water column (Vick-Majors et al, 2015) and sediments (Carr et al, 2013) beneath the McMurdo Ice Shelf (MIS) and RIS. These studies have revealed microbial communities in the water column and sediments that are dominated by Proteobacteria and Bacteriodetes, including prevalent phyla such as Plactomycetes, Thaumarchaeota, Actinobacteria, and Chloroflexi. Although sequences affiliated with chemoautotrophic species were detected in both studies, the proximity of the sample sites to the ice shelf edge combined with the presence of large putative heterotrophic populations, was consistent with advected organic matter supporting the ecosystem (Vick-Majors et al, 2015; Carr et al, 2013). Combined, these studies suggest that the significance of advected phytodetritus should diminish with distance from the ice edge, thus chemoautotrophic processes would dominate beneath the ice shelf.

In January of 2015, a hot water drill was used to cleanly access (Priscu et al, 2013; Rack et al, 2014) the grounding zone of the Whillans Ice Stream (WIS) at the southwestern head of the RIS providing the opportunity to make observations and recover samples. The WIS grounding zone (WIS-GZ) is located on the Siple Coast of West Antarctica, ~650km from the RIS margin in one of the most southerly embayments of the sub-ice shelf marine cavity. During episodic drainage events, active subglacial lake systems in this region transport large volumes of water ($300 m^3 s^{-1}$) from the interior of the

continent, across the grounding line, and into the Ross Sea (Fricker and Scambos, 2009; Carter and Fricker, 2012). Subglacial Lake Whillans, an active lake system beneath the WIS (Tulaczyk et al, 2014; Christner et al, 2014) that contains chemosynthetic bacterial and archaeal communities (Christner et al, 2014; Achberger et al, in draft), is known to have a drainage path towards the WIS-GZ site and was a primary consideration in the drill site selection. Previously, there have been no observations on the microbial community structures at RIS grounding zones nor have there been opportunities to examine the effect of subglacially derived runoff on the marine environments they discharge into.

Here, we present an analysis on the composition of bacterial and archaeal communities in water column and sediment samples collected from the WIS-GZ during the WISSARD (Whillans Ice Stream Subglacial Access Research Drilling) project. Analysis of amplified 16S rRNA genes (rRNA) and molecules (rRNA) allowed identification of the community structure and potential metabolically active members, respectively. Many of the taxa abundant in the communities closely resembled those of deep sea environments that rely on reduced sulfur species for lithotrophic metabolisms. When compared to microbial communities in SLW (Christner et al, 2014; Achberger et al, in draft) and the sub-MIS water column (Vick-Majors et al, 2015), the WIS-GZ was unsurprisingly unique. Our data provide information on the trophic foundation of an ecosystem in the most southerly reaches of the marine environment and possible influences in shaping and supporting the observed microbial and macrofaunal species are explored. Furthermore, this study also provides a baseline with which to compare possible future ecological shifts under the pending threat of climate change and Antarctic ice shelf collapse.

Materials and Methods

Site Description

The WIS, located along the Siple Coast in West Antarctica is one of five ice streams that supply ice to the RIS from the West Antarctic Ice Sheet (Carter and Fricker, 2012). At the base of these ice streams, active hydrologic features exist which transport water and sediments to the grounding zone

environment and influence ice sheet dynamics (Fricker and Scambos, 2009; Carter and Fricker, 2012; Horgan et al, 2013). In January of 2015, the ice overlying the grounding zone at the WIS was penetrated using a hot water drill at a location ~5 km (84.33543S, 163.6119W) from the physical grounding line of the ice stream and ~650m from the open Ross Sea (Blythe et al. 2014; Burnett et al. 2014; Rack et al. 2014). The ~720m borehole provided access to a ~10m water cavity and underlying sediments for ~11 days. Although the region is known to receive periodic inputs of subglacial material from beneath the WIS and neighboring Kamb Ice Stream (Fricker and Scambos, 2009; Carter and Fricker, 2012; Horgan et al, 2013), the water column showed no significant freshwater component at the time of sampling (conductivity = ~5 S m^{-1}).

Hot Water Drilling and Microbial Monitoring

Microbial contamination in the drilling water was minimized using technology and procedures that were extensively tested (Priscu et al, 2013) and used successfully at SLW in January 2013 (Christner et al, 2014; Achberger et al, in draft). The water decontamination involved sequential passage through large capacity filters (2.0µm and 0.2µm pore sizes) and irradiation with UV (185 and 245 nm) to remove and kill cells, respectively. Subsequently, the cleaned water was heated to ~90˚C and pumped through the hose for drilling in the borehole. Samples of the drill water were collected from access ports in the drill system at three designated time points [when drilling was at depths of ~80 mbs (Time point 1; T1), ~680 mbs (T2), and 714 mbs (T3)]. Drill system water samples (~20L) were obtained before filtration and UV treatment (Port 1) and following heating in the boilers, prior to entering the drill hose and borehole (Port 8). The final sample (T3) at 714 mbs was taken prior to breakthrough into the sub-ice marine cavity (ice thickness of ~720 m). An additional 10 L borehole water sample was obtained post breakthrough via hydrocast at 350 mbs for comparison.

The water samples were processed immediately following collection by filter concentrating the suspended cells onto 142mm, 0.2µm Supor membrane filters (Pall Corp.). Material retained on the

filters was preserved in either 5 mL of RNAlater (Ambion) or DNA preservation solution (40mMEDTA pH8.0, 50mMTris pH8.3, 0.73M sucrose). The samples were frozen for transport and stored at -80^{o}C in the laboratory until analyzed.

Water Column and Sediment Sampling

Planktonic cells and suspended particulates in the water column at the WIS-GZ were collected by sequential filtration onto Supor membrane filters (Pall Corp.) with 3, 0.8, and 0.2 μm pore sizes using a modified WTS-LV (McLane Research Laboratories Inc.), as previously described (Christner et al. 2014). There were four casts to the middle of the water column, with each filtering for 4-5 h. During the first cast, ~196L of water was filtered, while 324L and 73L were filtered during the second and fourth casts, respectively. Due to a technical issue with the flow meter, the volume sampled during cast three is unknown, but comparison of particle loads on the filters closely resembled those from the other casts. A 12 cm sediment core was recovered and sampled as previously described (Christner et al. 2014). The filters and sediment samples were processed and preserved for DNA and RNA extraction procedures as described in Achberger et al. (in draft).

Nucleic Acid Extraction and Phylogenetic Analysis

DNA was extracted from portions of the filters (1/8th of a 142mm filter) and sediment samples (0.5 g) using the Power Water DNA Isolation Kit and Power Soil DNA isolation kit (MO BIO Laboratories, Inc.), respectively, and according to the manufacture's protocol. RNA extraction from the filters and sediments (Dieser et al. 2014) followed by reversed transcription (Achberger et al., submitted) was conducted as previously described. DNA and RNA extraction blanks were processed together with the samples to serve as methodological controls. Amplification of the 16S rRNA gene V4 region (515F and 806R; Caporaso et. al. 2012) was performed as described in Achberger et al (in draft). The amplicons were pooled in equimolar concentrations and sequenced on an Illumina MiSeq (Michigan State RTSF Genomics Core).

Paired end sequence reads (250 bp) were assembled into contigs using the mothur phylogenetic pipeline (v1.33.3; Schloss et. al. 2009). Sequence data from the WIS-GZ were joined with the SLW (Achberger et al, in draft) and MIS (Vick-Majors et al, 2015) datasets to facilitate comparison. The compiled data were screened for quality and aligned using the SILVA database (v119). Chimeric sequences were identified and removed using the Uchime algorithm (Edgar et al. 2011) as implemented within mothur. Sequences that shared ≥97% sequence similarity were clustered as an operational taxonomic unit (OTU) and classified using the mothur compatible SEED database (v119). The taxonomic classification of representative sequences from each OTU were further evaluated through classification with the NCBI Genbank database. Estimations of diversity and richness (Shannon, Inverse Simpson, and Chao1) were performed in mothur on subsampled data. Prior to further analysis, singletons were removed and samples were normalized to the smallest sample (25,754sequences) and relative abundances were calculated. Abundant OTUs were defined as those that represent ≥1% of the total sequences in a given sample. Abundant OTUs in the drilling and extraction controls were considered potential contaminants and were eliminated from the WIS-GZ community description.

Ordination and statistical analysis was performed in R (v.3.1.2; R Core Team 2014) using the Labdsv (Roberts, 2007) and Vegan (Oksanen et. al., 2015) packages. Nonmetric multidimensional scaling (NMDS) analysis was computed on logarithmically transformed data and Bray-Curtis dissimilarity matrices. Statistical comparisons of community structure were performed using Adonis and analysis of similarity (ANOSIM).

Results

Comparison and Composition of the 16S rDNA and rRNA Data

Sequence data generated from 16S rRNA and rRNA gene amplicons from samples of the water column (cast 1-4) and 12 cm sediment core were compared to procedural control samples (i.e., drill system water, the borehole hydrocast, and the nucleic acid extraction blanks). Statistical analysis

revealed that the structure of the WIS-GZ communities was significantly different from the assemblage in the drilling water or borehole (p≥0.001) with the WIS-GZ samples grouping distinctly from the controls in nonmetric multidimensional scaling (NMDS) analysis (Figure 4.1A). The two methodological

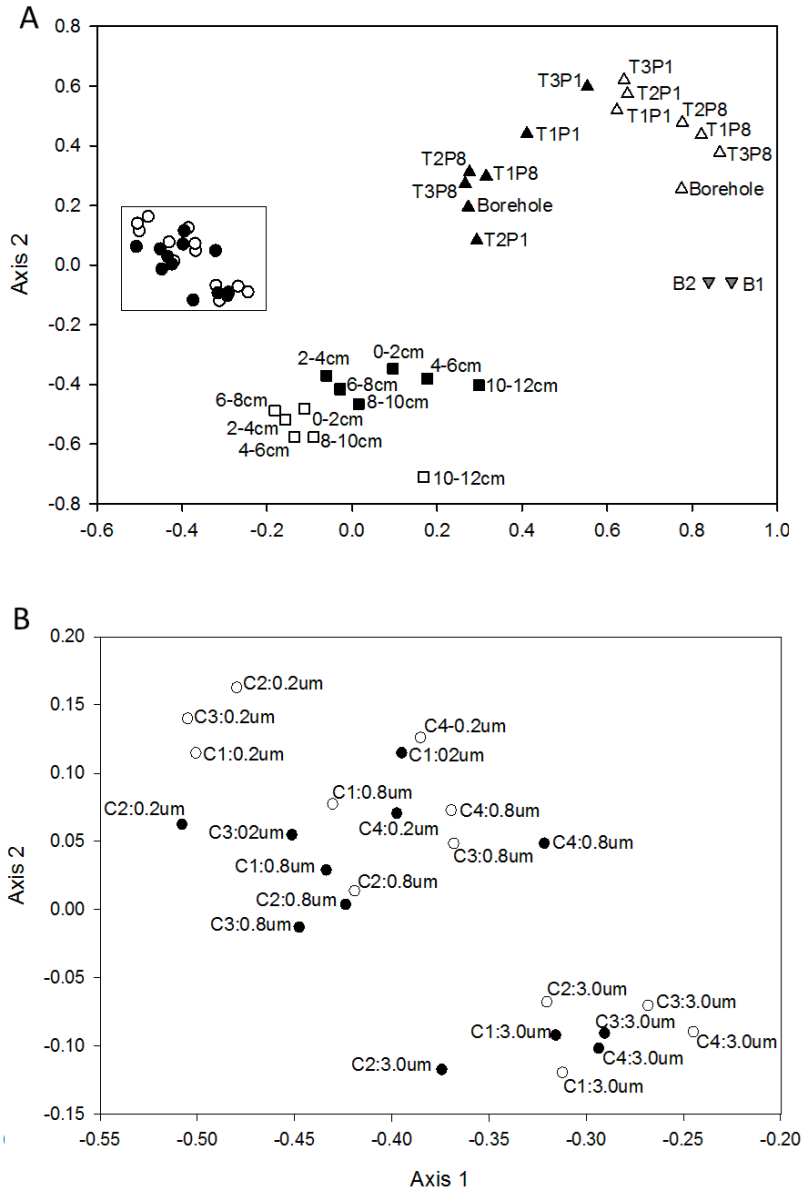


Figure 4.1: Nonmetric multidimensional scaling plot based on OTUs from the 16S rRNA and rRNA gene sequences that were obtained from (A) samples of the drilling water, WIS-GZ water column, sediments, and experimental controls. Panel B highlights the distribution of the 3.0, 0.8, and 0.2 um samples from each water cast outlined with a black box in panel A. Circles denote water column samples, squares represent sediment samples, triangles represent drilling water, and upside triangles are experimental blanks. Black symbols denote rRNA based libraries and white denote rDNA based libraries.

blanks generated during the extraction of 16S rRNA were also significantly different from the WIS-GZ communities (p≥0.003; Figure 4.1). Methodological blanks (n=4) processed during 16S rDNA extraction showed no visible amplification on an agarose gel. Sequencing of these samples resulted in a low number of reads (average=634 sequences) and they were therefore excluded from further analysis.

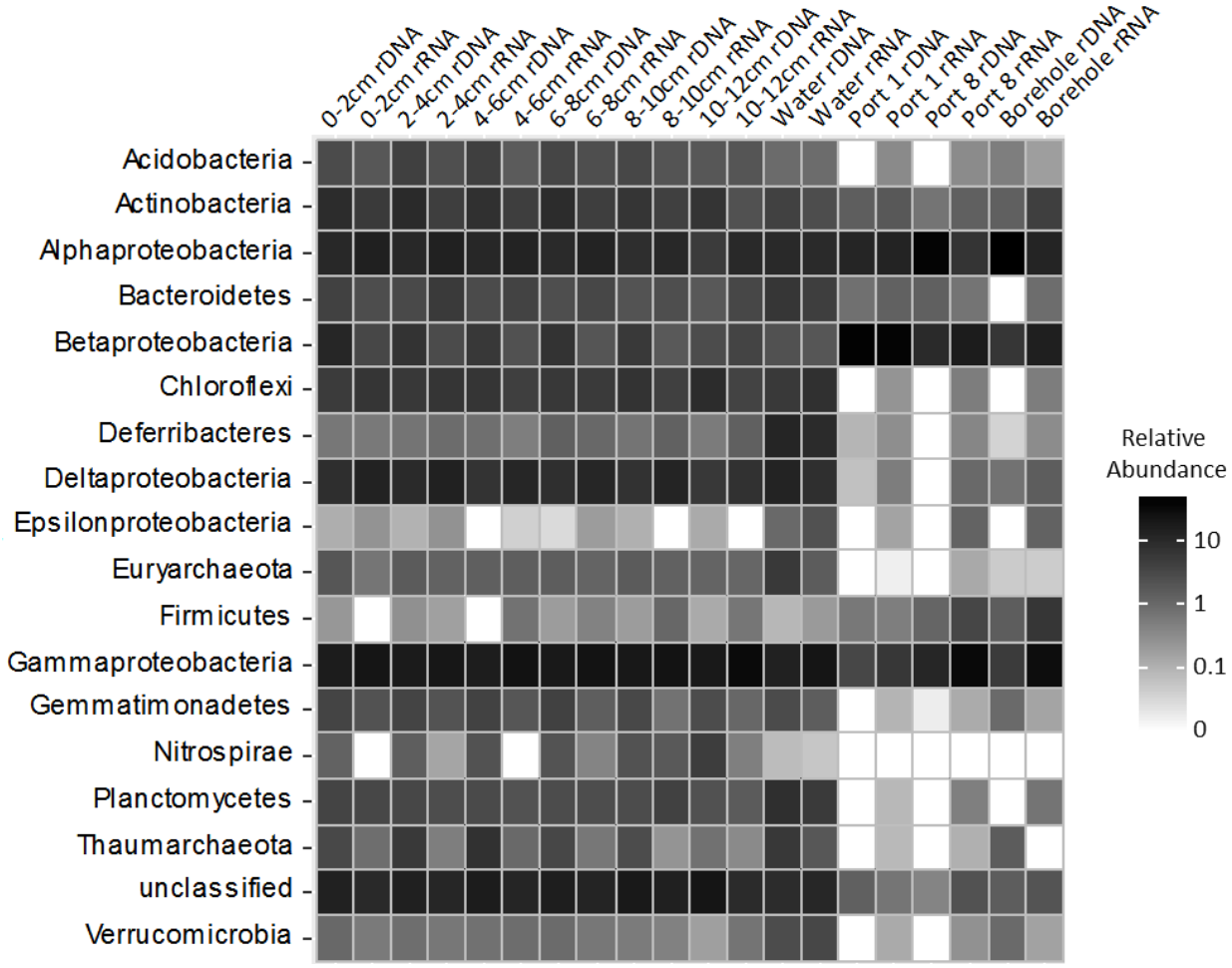


Figure 4.2: The relative abundance of the most abundant phyla (≥ 1%) in the WIS-GZ sediments, water column, drill water and borehole.

Microbial community composition and OTU abundance of the four water column casts was highly similar (p≥0.991) and grouped predominantly based on filter size fraction, with the smaller fractions (0.8 µm and 0.2 µm) occupying distinct NMDS space from the 3.0µm samples (Figure 4.1B). Differences in indices that describe community diversity (Inv. Simpson, Shannon) were also observed

between the size fractions, with diversity typically decreasing with the size fraction (Supplemental Table C.1). A similar trend was also found in the number of observed OTUs though it was not reflected in the Chao1 estimates of richness (Supplemental Table C.1). Although no trend was observed between the richness or diversity of the sediment community versus depth, the diversity observed at depths of 8-12 cm was lower than that in the upper horizons (0-8cm; Supplemental Table C.1).

Drill water samples were collected at three time points and from two locations in the drilling system over the course of drilling into the sub-ice shelf marine cavity. The microorganisms in water from Port 1 (P1; before filtration) were dominated by Betaproteobacteria (67.7-95.1% rDNA; 53.8%-94.2% rRNA; Figure 4.2), the abundance of which increased over the course of drilling by 27% and 40% in the rDNA and rRNA, respectively (Supplemental Figure C.1). Gammaproteobacteria and Alphaproteobacteria were also abundant in samples from Port 1 (Figure 4.2), but decreased over time in rDNA (rRNA) derived libraries from 24.1% (30.3%) to 4.28% (5.42%) and 4.84% (11.67%) to 0.17% (0.26%), respectively (Supplemental Figure C.1). The microbial assemblages in water entering the borehole (Port 8) did not show any trend over time; however, significant differences were observed in phylum abundances in the rDNA and rRNA based libraries. For example, the Alphaproteobacteria were dominant in the rDNA dataset (~75%) but were only ~6% of the rRNA sequences (Figure 4.2). Conversely, Betaproteobacteria and Gammaproteobacteria represented a higher percentage of the rRNA data (~15% and ~51%, respectively; Figure 4.2). Similar patterns were also observed in water that was collected in the borehole at 350 mbs, which had a community composition that closely resembled those observed from the Port 8 samples (Figure 4.2). The phylotypes identified in the extraction blank controls were predominantly composed of Gammapoteobacteria (55.3%; e.g., *Escherichia* and *Pseudomonas*) with Alphaproteobacteria and Betaproteobacteria making up a further 20.4% (e.g., *Phyllobacterium*, *Janthinobacterium*, and *Herbaspirillum*).

Composition of Bacteria and Archaea Abundant at the WIS Grounding Zone

The WIS-GZ planktonic and sedimentary microbial communities characterized through analysis of 16S rRNA and rDNA were found to be dominated by bacteria, with archaeal taxa constituting 3.48% of the rDNA based sediment (0.62% rRNA) and 8.96% (1.47% rRNA) of the water column sequences (≤2.7% of the OTUs were unclassified). Archaeal OTUs that classified as Thaumarchaeota and Euryarcheaota were equally represented in the water column at 4.56% (0.82% rRNA) and 4.38% (0.63% rRNA) of the rDNA sequence data, respectively, (Figure 4.2). Thaumarchaeota were more abundant than the Euryarchaeota in the sediment community based on the rDNA library (2.74% vs. 0.64%), with lower representation in the rRNA data (0.16% vs. 0.42%; Figure 4.2). The most abundant Euryarcheaotal OTUs were related to poorly classified members of Marine Group I and II, while the Thaumarchaeotal OTUs were widely distributed in the WIS-GZ samples and shared ≥97% identity with *Nitrosopumilus maritimus* (OTUs 000830, 003830; Figures 4.2 and 4.3).

Gammaproteobacteria were the most numerically dominant bacterial taxa detected in the WIS-GZ communities (Figure 4.2), with numerous OTUs most closely related to *Thioprofundum lithotrophicum* (≥94% identity; OTUs 000033, 000628, 001134, 001365, 002040) and *Thioprofundum hispidum* (≥93% identity; OTUs 001014, 001297, 028737; Figure 4.3) being abundant. Together, these eight OTUs represent ~13.0% of the sediment rDNA sequence reads (5.9% rRNA) and 1.5% of those obtained from the water column (8.9% rRNA). Also prevalent within the sediments were OTUs most closely related to *Thiobacillus denitrificans* (OTU000064; 98% identity), *Methylobrevis pamukkalensis* (OTU000196; 96% identity), and *Methylovulvum miyakonense* (OTU008881; 94% identity). The majority of OTUs in the sediments showed a spatial preference within the core, either increasing (e.g. OTUs 00881 and 028737) or decreasing (e.g. 000670) in abundance with depth (Figure 4.3).

Many of the most prevalent water column OTUs are poorly classified members of the Chloroflexi (OTU000039; 1.5% rDNA and 3.7% rRNA), Deltaproteobacteria (OTU000004; 9.6% rDNA and 1.1% rRNA),

and the Deferribacteres (OTU000178; 3.4% rDNA and rRNA). Several OTU sequences share ≥93% identity to *Thiohalophilus thiocyanatoxydans* (OTUs 000152, 000456, 000901, 006234) and together represented 1.3% of the rDNA based community and 8.8% of the rRNA (Figure 4.3; Supplemental Table C.3). Although many numerically dominant water column taxa were present in samples from the three filter size fractions, they were often most prevalent on the smaller 0.8 and 0.2 µm fractions (Figure 4.3), while sediment taxa detectable in the water column were frequently found in the 3.0 µm pore size samples (e.g., OTU000064, 008881).

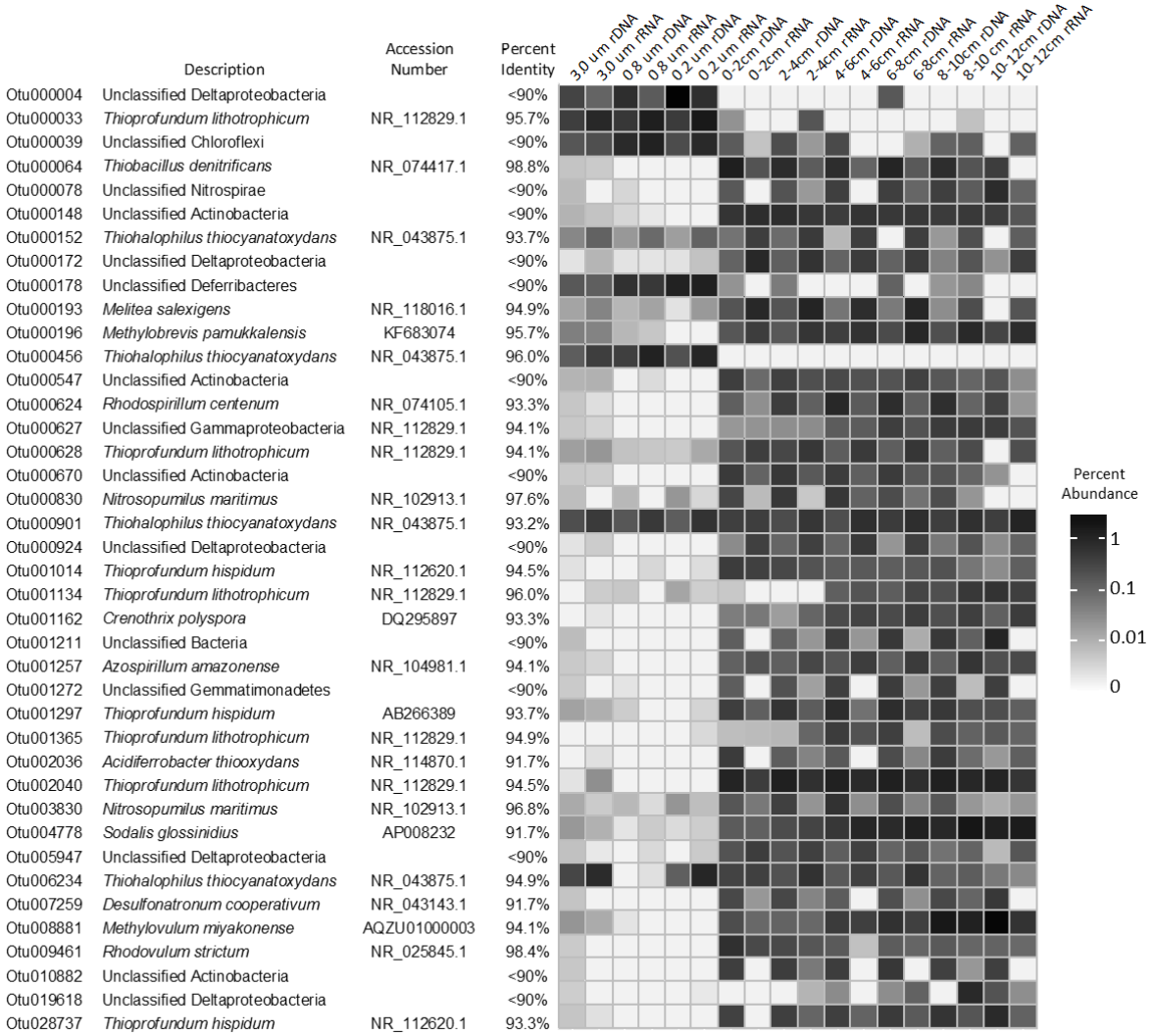


Figure 4.3: The 40 most abundant OTUs in the WIS-GZ water column divided according to filter size fraction and sediment depth. All of the OTUs are listed with their nearest taxonomic neighbor.

Comparative Analysis of the 16S rRNA and rDNA Sequence Data

The ratio of 16S rRNA to rDNA sequences for each OTU was examined as an indicator of potential metabolic activity (e.g., Blazewicz et al, 2013). In the WIS-GZ communities, 48.9% of the OTUs in the rRNA and rDNA derived libraries had an rRNA:rDNA ratio greater than 1, with ~6% being major outliers (ratio>7.6; Supplemental Figure C.2A). Of the major outliers, OTUs represented by at least 10 sequence reads in the rDNA based datasets were examined in detail. Many of the numerically dominant taxa within the WIS-GZ community also had high rRNA:rDNA ratios, including OTUs most closely related to *Thiohalophilus thiocyanatoxydans* (OTUs 000152, 000525, 000591, 006234), *Thioprofundum hispidum* (OTU 000628), *Melitea salexigens* (OTU 000172), *Azospirillium amazonense* (OTU 001257), and unclassified Deltaproteobacteria (OTUs 000172, 000924; Figure 4.3 and Supplemental Figure C.2B). The *Thiohalophilus*-related OTU 000152 had the largest ratio (287) and was found throughout the water column and upper 12 cm of sediments (Figure 4.3 and Supplemental Figure C.2B). OTUs 0001221 and 000704 also had large ratios of (111 and 95, respectively) and were rare community members that were most abundant at depths of 8-12 cm in the sediments (Supplemental Figure C.2B).

Comparison of the WIS-GZ, SLW, and MIS Microbial Communities

The microbial community structure of the WIS-GZ was compared to subglacial (i.e., SLW) and marine endmember systems (i.e., MIS) both previously investigated as part of the WISSARD project (Figure 4.4A). Inverse Simpson and Shannon indices indicated that the WIS-GZ had the highest species diversity and richness of the three sites (Supplemental Table C.2). Despite having the fewest observed OTUs, the SLW water column community was more diverse than those sampled at the MIS (Supplemental Table C.2).

Analysis of the 16S rRNA and rDNA data revealed that the microbial composition at the three sites was statistically different and they group distinctly in a NDMS analysis ($p \geq 0.001$; Figure 4.4B). Several differences are also evident based on the phylum level distribution of sequences from the water

column communities at the three sites. Notably, larger populations of Actinobacteria, Lentisphaerae, and Betaproteobacteria were inferred at SLW while the marine WIS-GZ and MIS communities contained more Gammaproteobacteria, Deferribacteres, and Euryarchaeota (Figure 4.5). Despite these difference, the WIS-GZ community more closely resembled that at the MIS (Figure 4.4B), sharing ~8% of their OTUs (63% of sequences, Figure 4.6). The majority of the OTUs shared between the sites were rare taxa within the communities; however, poorly classified members of the Deltaproteobacteria (Otu000004), Gammaproteobacteria (Otu000224), Deferribacteres (Otu006784), and Euryarchaeota (Otu000017) as well as OTUs most closely related to *Illumatobacter coccineus* (91% Identity; Otu000108), *Owenweeksia hongkongensis* (92% Identity; Otu000233), and *Candidatus* Vesicomyosocius okutanii (96% Identity; Otu021440) were abundant at both marine sites (Supplemental Figure C.3). Although the WIS-GZ and SLW water columns shared less than 1% of their OTUs (~13% of sequences), ~3% (38% of sequences) of the sediment OTUs were found at both locations (Figure 4.6). Of those, an OTU sharing 98% identity to *Thiobacillus dentrificans* (Out000064) and a poorly classified member of the Nitrospirae (Otu000078) were numerically dominant within both sediment communities (Supplemental Figure C.3).

Discussion

The properties of ice shelf grounding zones are recognized as integral features in the dynamics of ice shelves and sheets (Goldberg et al, 2009; Pritchard et al, 2012; Rignot et al, 2013). Logistical constraints have prevented extensive exploration of these environments, with remote sensing and surface measurements providing the bulk of what is known about their position and characteristics (Fricker et al, 2009; Brunt et al, 2012; Christianson et al, 2013). In January of 2015, the WISSARD project drilled through ~720m of the RIS in a location that provided direct access to the seaward cavity in proximity (5 km) to the WIS grounding zone. This effort enabled physical and oceanographic characterization of the sub-ice shelf environment, and the unprecedented opportunity to examine an Antarctic ecosystem at the southern limits of the marine biosphere.

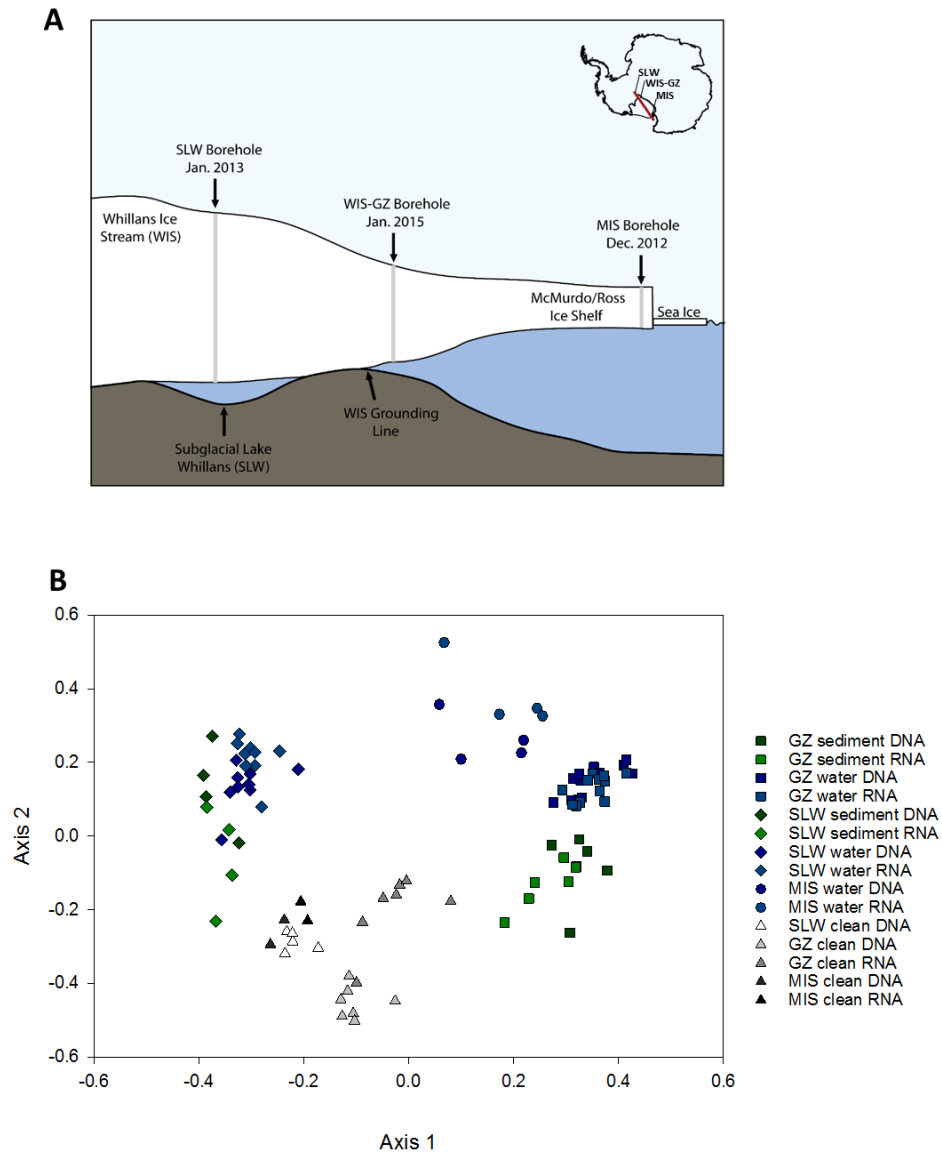


Figure 4.4: WISSARD site comparison based on 16S rRNA and rDNA analysis. A) Schematic representation of study sites. B) Nonmetric multidimensional scaling plot based on OTUs from the 16S rRNA and rRNA gene sequences that were obtained from samples of the drilling water, water column and sediments from MIS, SLW, and WIS-GZ.

Real time video observations of the shallow water column of the WIS-GZ revealed a sparse population of fish, crustaceans (e.g., amphipods), and other marine invertebrates. Although taxonomic classification of these organisms is pending, initial observations suggest that they are similar to species commonly observed beneath the calving fronts of the McMurdo and Ross Ice Shelves. No sessile

organisms were observed within the proximity of the borehole and investigations into the diversity and abundance of micro-eukaryotic populations within the ecosystem has not yet been conducted.

The composition of the WIS-GZ bacterial and archaeal communities could be confidently accessed through the comparison of samples to drilling water and methodological controls. The WIS-GZ water column and sediment communities were phylogenetically diverse, composed chiefly of Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Actinobacteria, Chloroflexi, and Deferribacteres (~61% rDNA and ~76% rRNA; Figure 4.2). In contrast, water sampled from the drilling system consisted almost exclusively of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria (≥94%). The source water for the drilling system (Port 1) became enriched in Betaproteobacteria over time (Supplemental Figure C.1). This was largely due to an increase in the abundance of a single *Massilia*-like OTU, which represented ~30% of the sequence reads in the first time point but was ~83% of rDNA reads (~74% rRNA) prior to the completion of drilling. *Massilia* species are commonly detected in atmospheric (Weon et al, 2008 and 2009), aquatic (Gallego et al, 2006), and terrestrial (Zhang et al, 2006) environments, including Antarctic soils (Wery et al, 2003). Its large contribution to the data over time is consistent with elevated abundances in deeper portions of the ice sheet, which sourced the hot water used to create the borehole (Blythe et al, 2014; Burnett et al, 2014; Rack et al, 2014). Water that was pumped down the borehole (Port 8) showed no temporal variability, however Gammaproteobacteria and Betaproteobacteria were more highly represented in the rRNA based community than in the rDNA (Figure 4.2). The most abundant such OTUs were related to *Escherichia-Shigella* and *Herbaspirillium* sp., which together represented up to 50% of the rRNA sequence reads. These taxa were also among the most abundant observed in the rRNA extraction blanks (~44%), implicating them as contaminants that were likely introduced during sample processing, amplification, and or sequencing.

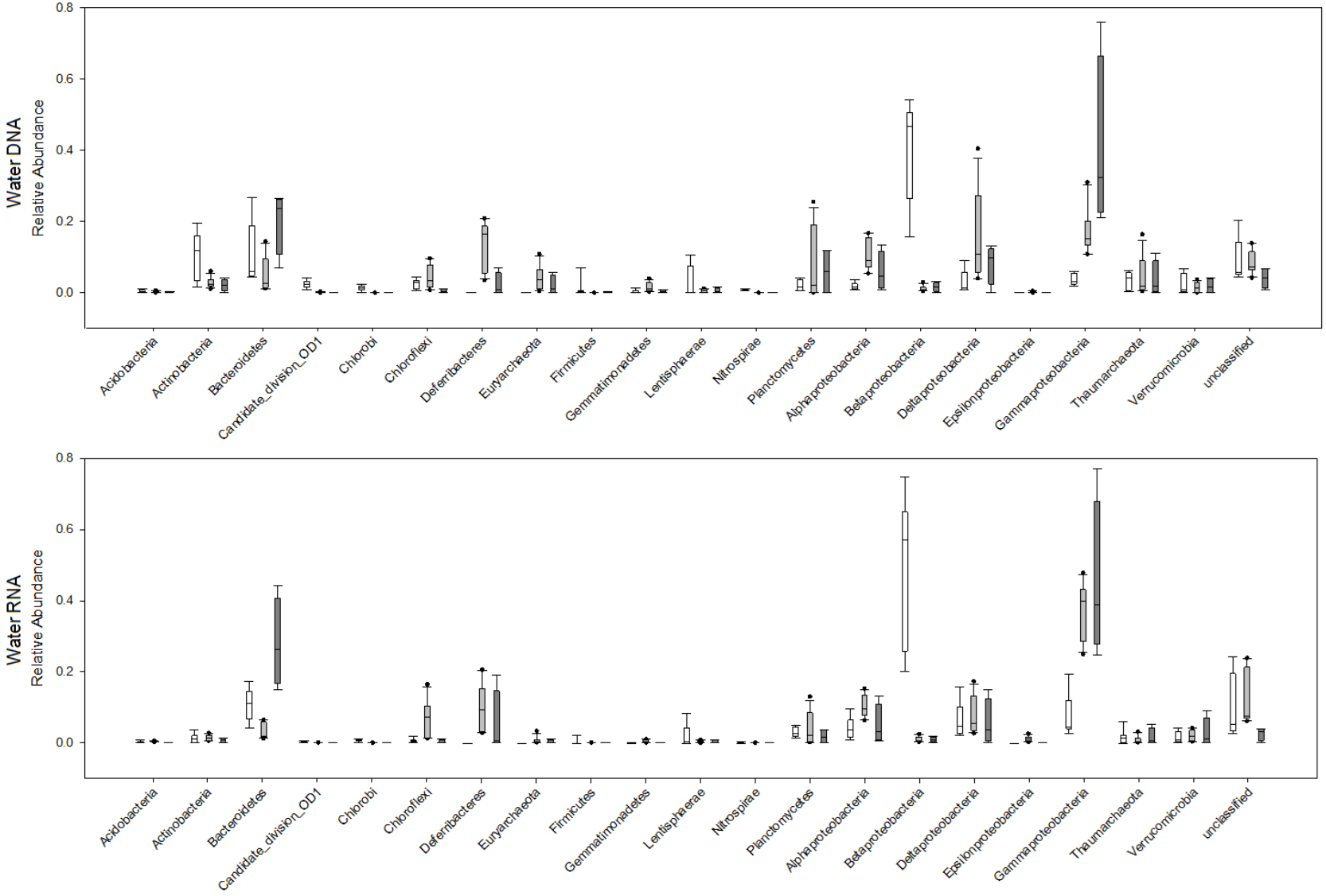


Figure 4.5: The relative abundance of the most abundant phyla (≥ 1%) in the water columns of SLW, WIS-GZ, and MIS. SLW, WIS-GZ, and MIS are denoted by white, light grey, and dark grey boxes respectively.

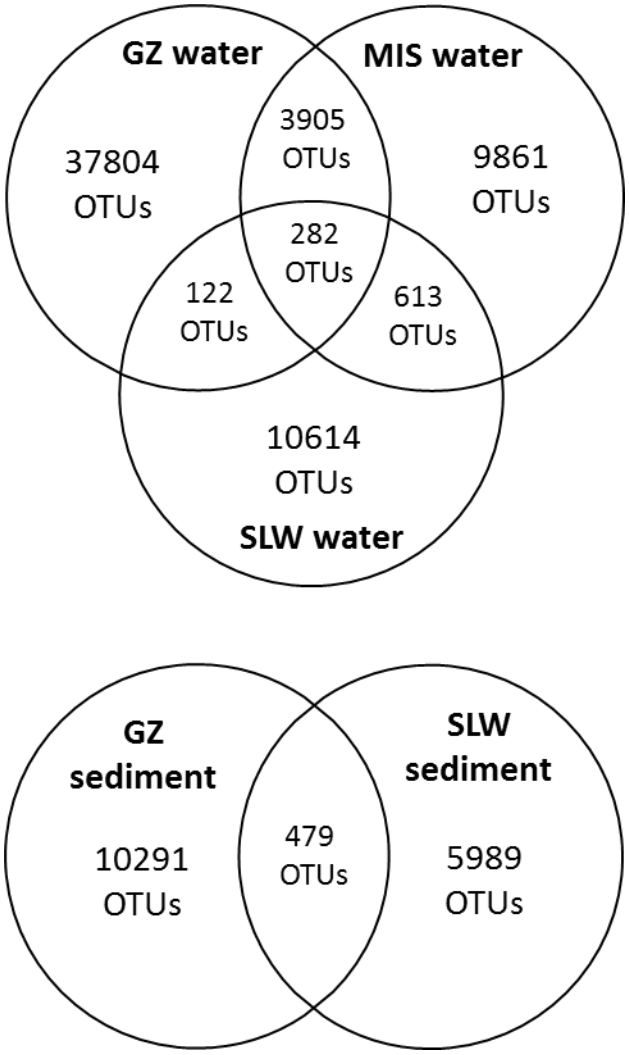


Figure 4.6: Comparison of taxa present at SLW, MIS, and WIS-GZ. Venn diagrams showing the number of shared OTUs between water column and sediment samples from SLW, MIS, and WIS-GZ.

Many of the abundant OTUs observed in the WIS-GZ ecosystem were most closely related to bacteria capable of lithotrophic growth using reduced sulfur species (Figure 4.3). Both *Thioprofundum lithotrophicum* and *Thioprofundum hispidum* are obligate chemolithoautotrophs that were isolated from marine hydrothermal fields (Takai et al, 2009; Mori et al, 2011) and are frequently observed in seafloor basalt communities (Lee et al, 2015). The nearest neighbor of the most abundant OTUs in the water column was *Thiohalophilus thiocyanatoxydans* (Sorokin et al, 2007), which also possessed the highest rRNA:rDNA ratio observed (Supplemental Figure C.2B). *T. lithotrophicum, T. hispidum*, *T. thiocyanatoxydans*, and *Thiobacillus denitrificans,* another sulfur oxidizing bacteria (SOB) similar to OTUs

abundant at the WIS-GZ, all possess the ability to grow aerobically or anaerobically using nitrate or nitrite as an electron acceptor. This versatility may explain the prevalence of these taxa throughout the water column and sediments (Figure 4.3) where variability in redox conditions would be expected. The oxidation of reduced sulfur compounds (e.g., hydrogen sulfide, pyrite, etc.) has been found to be a key source of energy in a number of deep sea environments, particularly in basalts, which fuels microbial carbon fixation in the absence of photosynthetically available radiation (Edwards et al, 2005).

Despite a dearth of data comparing the abundance of 16S rRNA molecules to that of 16S rRNA genes for individual taxa in nature (Blazewicz et al, 2013) and the know variability of ribosome concentrations between species (Lankiewicz et. al., 2015), rRNA:rDNA ratios have been applied as a means of identifying members of a community with the potential for metabolic activity (e.g., DeAngelis et al, 2010; Jones and Lennon 2010; and Campbell et al, 2011). The large ratios exhibited by SOB-like taxa are interpreted as signatures consistent with active metabolism in the water column and sediments (0-10 cm; Supplemental Figure C.2B) at the time of sampling. The abundance of SOB populations suggest sulfate reduction may provide substrate to sustain their lithotrophic metabolism. Although known sulfate reducing species were generally rare in the sediment rRNA and rDNA data, they may be abundant and active at depths greater than those examined for this study (0-12 cm).

The ability to oxidize C1 compounds such as methane could be an important characteristic of the WIS-GZ that is shared with other sub-ice shelf and subglacial (Dieser et al. 2014; Achberger et al., submitted) microbial communities. Although there are no methane data from beneath the RIS, methane hydrates occur widely in marine sediments (Milkov, 2004) and numerous active methane seeps have been documented in the southern ocean (Romer et al, 2014). Indeed, cold seeps are hypothesized to have supported chemosynthetic communities beneath the former Larson B Ice Shelf (Domack et al, 2005; Hauquier et al, 2011) and biological methane sources have been documented beneath the WAIS (Michaud et al, submitted). OTUs related to *Methylobrevis* (OTU000196),

Methylovulum (OTU008881), and *Crenothrix* (OTU001162) were abundant in the sediments, particularly deeper horizons (8-10 cm) where they together represented ~12% of the rRNA sequence data. (~15% rDNA; Figure 4.3). The presence of these taxa suggests that oxygen is present several cm below the water-sediment interface and that sources of C1 compounds (e.g., methane and methanol) may exist in the sediments or water column (e.g. Mincer and Athena, 2016).

The data also support the presence of a small, potentially active population of Thaumarchaeota in the water column and upper sediments (0-8cm; Figure 4.3). This combined with the presence of nitrite oxidizing *Nitrospina* species (~0.1% rRNA), suggests nitrification may occur in the water column at the WIS-GZ. Thaumarchaeota are widespread in polar waters (Kalanetra et al, 2009; Alonso-Saez et al, 2011; Vick-Majors et al, 2015) where primary production fueled by ammonia oxidation is a significant source of fixed carbon during winter months (Tolar et al, 2016). Nitrification was also hypothesized to drive carbon fixation at site J9 on the RIS where data suggest that chemolithoautotrophic production was sufficient to sustain the observed population of macrofauna (Horrigan, 1981).

Many of the abundant OTUs (Figure 4.3) identified in the WIS-GZ ecosystem had relatively low phylogenetic relatedness to cultured or typed species (Figure 4.3). Such OTUs showed greatest similarities to sequences that were recovered during molecular surveys of marine waters and sediments (OTUs 000172, 000148, 005947, 000924, and 000004; Li and Wang 2014; Allers et al, 2013; Li et al, 2013; Wu et al, 2013), including cold seeps (OTU 000078; Heijs et al, 2007) and crustal fluid (OTUs 000178, 000627, 019618; Santelli et al, 2008). Of these, two (OTUs 000172 and 000924) were among the OTUs with the largest rRNA:rDNA ratios (Supplemental Figure C.2B), supporting their potential activity and importance to biogeochemical transformations in the WIS-GZ environment. Although the functional contributions of these species are not discernable, their similarity to other deep sea marine microorganisms points toward the commonality between these ecological settings (e.g., lack of sunlight

and low temperature and nutrients) and plausible source of progenitors for some members of the WIS-GZ community.

The reliance of sub-ice shelf marine communities on autochthonous organic carbon and nutrients versus those advected from open water is unclear as few observations from such environments exist (Horrigan, 1981; Riddle et al, 2007; Vick-Majors et al, 2015; Carr et al, 2013). The residency time of water beneath the RIS is poorly constrained, with predicted circulation times of 3-8 years for water masses that enter the ice-shelf cavity (Loose et al, 2009; Smethie and Jacobs, 2005; Holland et al, 2003). This means best case estimates for oceanographic delivery of new material to the WIS-GZ is on the order of years. Sparse phytoplankton were documented beneath the RIS at the J9 station (~400km; Holm-Hansen et al, 1978) however, the extent of their transport beneath the RIS and their suitability as a carbon source is unknown. Based on this information, transported phytodetritus is unlikely to be a sufficient source of organic carbon and energy to support ecosystem processes in the grounding zone region of the Ross Sea. The WIS-GZ ecosystem may however be supported in part by subglacial outflow along the Siple Coast. Based on data from SLW, such outflow could transport enough dissolved organic carbon to sustain a substantial fraction of the heterotrophic demand (average ~20%; Vick-Majors et al, in draft). The relative importance of advected, subglacially derived, and chemolithoautotrophic organic carbon sources to sub-ice shelf ecosystem production warrants more detailed investigation.

A high degree of similarity between the WIS-GZ and MIS communities (versus WIS-GZ and SLW) is indicated by compositional analysis (Figure 4.4) and OTU level comparisons (Figure 4.6). Although less than a tenth of OTUs were shared between the WIS-GZ and the MIS water columns, they represented over half of the total sequence reads for the groups suggesting that the marine waters flowing beneath the RIS are a major source of the WIS-GZ water column microbial community. Given the temporal variability of subglacial outflows (Carter and Fricker, 2012), the contributions of subglacially derived

microbes transported through the WIS estuary may not have been pronounced at the time of sampling. However, a large portion of the WIS-GZ sediment OTUs and sequence reads were shared with those of the SLW sediment communities (Figure 4.6). The similarity between communities likely reflects the marine history of the WIS region (e.g., Scherer et al, 1998), and suggests that key species in the SLW ecosystem were derived from microbiota similar to those currently inhabiting the WIS-GZ.

Conclusion

The diversity and ecology of ecosystems beneath ice shelves is poorly understood due to a lack of direct observations. As changing climate conditions continue to threaten the stability of these regions, it become increasingly important to understand the biogeochemical characteristics of such environments and provide a baseline with which to evaluate pending ecosystem changes. The grounding zone at the WIS was the first to be explored for the RIS and was found to harbor diverse bacterial and archaeal species that compositionally resembled deep sea chemosynthetic communities that derives energy from sulfide and methane oxidation. Consistent with observations near the edge of the RIS (Priscu et al, 1990) and at the J9 station (Horrigan, 1981), nitrification may also be an important chemolithoautotrophic process beneath the RIS. The ability of *in situ* primary production to support the macro- and microorganisms beneath the ice shelf remains to be evaluated. Indeed, it is unclear if the macrofauna observed at the site even represent permanent members of the habitat as they may be transient to the system. Compared to SLW and the MIS site, the microbial communities of the WIS-GZ ecosystem were uniquely structured. Despite the clear differences in the community composition of the sites, large fractions of the WIS-GZ community were shared with the MIS and SLW, indicating their modern and historical connectedness, respectively.

CHAPTER 5.
CONCLUSION

The vast hydrologic network found beneath the Antarctic ice sheets is among the least explored regions of the subsurface biosphere. Although subglacial environments are now recognized to harbor a globally significant reservoir of microorganisms, the logistical challenges of directly and cleanly sampling these regions have rarely been overcome. Early attempts to investigate the ecology of Antarctic subglacial environments, particularly lakes, were limited by technology and plagued with concerns over contamination (e.g., Bulat et al, 2004; Inman, 2005; Alekhina et al, 2007; Chapter 1). As a consequence, little information is available concerning the distribution, diversity, and activity of microbial ecosystems beneath ice sheets. Motivated by this need, this study is the first to investigate the microbial ecology of an Antarctic subglacial hydrological system consisting of an active lake (Subglacial Lake Whillans; SLW) and the ocean basin in which it discharges (Whillans Ice Stream grounding zone; WIS-GZ).

Due to its location beneath ~800m of ice, SLW is isolated from sunlight and modern sources of nutrient input. The prevalence of putative chemolithoatutorophic taxa suggests that members of the microbial community are capable of serving as primary producers and supplying new organic carbon to the system in the absence of photosynthetically available radiation (Chapter 2). Indeed, laboratory experiments found that rates of inorganic carbon fixation were measureable in samples from SLW (Christner et al, 2014), and genes associated with various autotrophic pathways were detected (Chapter 3). In agreement with hypotheses generated based on 16S rRNA analysis (Chapter 2), the taxonomic affiliation of genes involved in aerobic carbon fixation (i.e., Calvin cycle) implicated species of *Thiobacillus*, *Nitrosospira*, and *Sideroxydans* as the major autotrophic organisms in the SLW system (Chapter 3). Together with the detection of genes for sulfide and ammonia oxidation also related to such taxa, this suggests that reduced sulfur and nitrogen compounds may be important energy sources driving primary production in SLW (Chapter 3). The oxidation of sulfide minerals (i.e., pyrite) in particular has been highlighted as a key microbial process occurring beneath ice masses (Chapter 1 and

references therein) and based on the abundance of genes involved in sulfide oxidation, it may be a dominant energy generation pathway in the SLW water column and surficial sediments (Chapter 3; Michaud et al, 2016).

The genetic potential for anaerobic carbon fixation in SLW (e.g., reductive tricarboxylic acid cycle) was greatest in the sediments and largely affiliated with organisms capable of sulfate reduction (Chapter 3). However, the sulfate concentration profile in the sediments of SLW did not suggest that sulfate reduction was an active process occurring at the time of sampling (Michaud et al, 2016), and consequently, anaerobic carbon fixation may not have been a significant contributor to primary production. Despite this, OTUs most closely related to sulfate reducing bacteria exhibited large rRNA:rDNA ratios (Chapter 2) and small but measurable rates of sulfate reduction were detected in laboratory microcosm experiments (Purcell et al, 2014). Additionally, modeling of the potential sources and sinks for oxygen within SLW suggest that the lake may experience periods of anoxia (Vick-Majors, in draft). Taken together, this could suggest that organisms within SLW experience significant environmental fluctuation and may maintain a physiological readiness to respond to conditions should they become favorable (Chapter 2; e.g., Blazewicz et al, 2013).

Relic marine organic matter deposits are thought to be an important source of carbon and other nutrients needed to sustain the SLW ecosystem (Chapters 2 and 3). While some of this pool may be labile and readily used by the microbial community, large portions are likely to be recalcitrant (Vick-Majors et al, in draft). The abundance of *Polaromonas* and *Rhodococcus*, which were found to have the genetic potential to degrade complex organic molecules (i.e., aromatic compounds; Chapter 3), may indicate the ecological importance of these organisms and their potential role in mineralizing recalcitrant compounds merits further investigation.

The marine based organic carbon stored beneath the West Antarctic Ice Sheet (WAIS) has also been predicted to fuel the production of methane, which trapped by the overburdening ice sheet, would

accumulate over time to a globally significant concentration that could have climatic implications if released to the atmosphere (Wadham et al, 2012). Geochemical and isotopic composition data have provided evidence for hydrogenotrophic methane in SLW although, the timeframe of its production is unknown (Michaud et al, submitted). Despite the fact that methane production was not directly measured, methanogenic archaea were detected in 16S rRNA based surveys from the deepest sediment depths sampled (Chapters 2 and 3) which could suggest their activity at the time of sampling and thus the contemporary production of methane beneath the WAIS. However, the abundance of taxa closely affiliate the methanotrophic species *Methylobacter tundripaludum* within the 16S rRNA datasets (Chapter 2) also indicates the potential for active aerobic methane oxidation to occur. In Greenland, a closely related *Methylobacter*-like taxa was similarly implicated in the oxidation of methane derived from subglacial water outflows, suggesting that this organism may play a key role in mediating the global release of methane from polar environments. This dissertation and associated studies (e.g., Michaud et al, submitted) are the first to present evidence for the microbial production and consumption of methane in an Antarctic subglacial environment, reinforcing the need to further evaluate the sources and sinks of methane to accurately predict the potential influence of polar environments to the atmospheric release of greenhouse gasses.

Aside from those related to methanogenic species, the majority of archaeal sequences found in SLW were affiliated with the ammonia oxidizing Thaumarchaeota (Chapters 2 and 3). This group has only been reported in one other subglacial setting (Robertson Glacier in Canada) where it was a minor component of the community and thought to be outcompeted by ammonia oxidizing bacterial (AOB) taxa (Boyd et al, 2011; Hamilton et al, 2013). In SLW, the predicted abundance of the *Thaumarchaeota* based on metagenomic sequencing was several fold higher than that of the 16S rRNA surveys (Chapters 2 and 3). This discrepancy may be attributed to the known 16S primer biases against this and other groups (Apprill et al, 2015), signifying that the *Thaumarchaeota* may be more prevalent in SLW than

originally thought (Chapter 2). Based on the relative abundance of the archaeal ammonia oxidizing (AOA) Thaumarchaeaota as compared to AOB, the AOA were hypothesized to outcompete the AOB as the dominant nitrifiers in the SLW water column (Chapter 2). Furthermore, the potential for the Thaumarchaeota in SLW to use urea in addition to ammonia is a feature that it shares with many deep marine polar species (Alonso-Sáez et al, 2012) and may provide a metabolic advantage in times of ammonia limitation. However, the taxonomic affiliation of genes involved in ammonia oxidation suggested that AOB may be the major nitrifiers in SLW (Chapter 3). Overall, the dynamics between AOA and AOB within the subglacial system remains unclear and merits further investigation.

The rarity of eukaryotic sequences in samples from SLW makes it difficult to evaluate their ecological importance to the subglacial food web, as it is possible that such sequences are the result of contamination or represent non-viable organisms (Chapter 3). Nevertheless, active eukaryotes have been found in subglacial environments (e.g., D'Elia et al, 2009; Hamilton et al, 2013), and the excess of organic carbon in SLW may be sufficient to sustain eukaryotic heterotrophs. However, like the prokaryotic population, they would expected to be limited by the availability of other key nutrients (e.g., nitrogen and phosphorous; Christner et al, 2014; Vick-Majors, 2016).

Given the dynamic hydrology of the WIS region, SLW may be a good model for the characteristics of aquatic environments along the Siple Coast of West Antarctica. At the time of sampling, SLW was in the process of filling (Siegfried et al, 2016), making it possible that the water and planktonic microorganisms in the lake were sourced from upstream tributaries (Carter et al, 2013). However, SLW is a relatively small feature in the overall Antarctic subglacial landscape, and it is unclear how representative its microbial community may be to that of other Antarctic lake systems. It is possible that larger subglacial lakes such as Vostok and Ellsworth with different hydrologic and geochemical attributes may have developed distinct microbial assemblages over prolonged periods of isolation (>400 kyr). Interestingly, many of the dominant species found in SLW were highly similar to

those reported in water saturated sediments beneath the neighboring Kamb Ice Stream (Lanoil et al, 2009; Christner et al, 2014) and in other polar and mountain glacial systems (e.g., Boyd et al, 2014; Dieser et al, 2014; Harrold et al, 2015; Chapters 2 and 3). The occurrence of related species in geographically distal subglacial systems suggests that such organisms are well suited for life in low temperature, low nutrient environments, and raises questions about the global distribution and evolution of polar microorganisms.

The WIS hydrological basin transports water and sediments to the GZ and marine environment beneath the Ross Ice Shelf (Carter and Fricker, 2012; Horgan et al, 2013), a region thought to receive few nutrients from the open Ross Sea and may be carbon limited (Church et al, 2000; Vick-Majors et al, 2015). In the absence of other sources, subglacial runoff may augment depleted nutrients (particularly DOC) and support ecosystem processes beneath the RIS (Chapter 4; Vick-Majors et al, in draft). Many of the dominant microbial species observed at the WIS-GZ were related to chemolithotrophic organisms that derive their energy from the oxidation of sulfur compounds such as thiosulfate (e.g., *Thioprofundum* and *Thiohalophilus*; Chapter 4). These and other abundant, yet poorly classified taxa most closely resembled organisms and sequences identified in deep sea environments, suggesting that the limited carbon input and their reliance on reduced chemical compounds may be dominant factors in shaping the community (Chapter 4). It is unclear if the macrofauna (e.g., fish, crustaceans, and comb jellyfish) observed at the WIS-GZ constitute permanent members of the system or if they are transient (Chapter 4). It is possible that carbon and nutrients rained out from the overlying ice sheet, transported in subglacial runoff, and advected from the open ocean may supplement the *in situ* chemolithoautotrophic production to sustain the tropically complex ecosystem of the WIS-GZ. However, the relative importance of these various sources remains poorly constrained.

Comparison of the microbial communities indicated that the WIS-GZ had more species in common with the MIS than SLW. This is unsurprising given the relatively minor contribution of

subglacial runoff to the overall water mass of the area and the salinity of the site, which would likely hinder the establishment of organisms from the freshwater subglacial system. Interestingly, the WIS-GZ and SLW sediments harbored more similar species than their water columns which may be indicative of their common history. The SLW region was linked to marine waters in the past (e.g., Scherer et al, 1998; Pollard, 2009), and was probably very similar to conditions at the modern WIS-GZ. Over time, the marine waters in the area would have been diluted by glacial melt and consequently seeded with microorganisms transported from inland subglacial sources and melted glacial ice. An investigation into the deeper sediments of the WIS-GZ and SLW (shown to have an increasing marine signature with depth; Michaud et al, 2016), combined with an evaluation of the inland source waters of SLW may yield insight into the geological and biological evolution of this dynamic region.

The work presented in this dissertation represents many firsts in Antarctic subglacial exploration and consequently, we are far from having a holistic understanding of the diversity and function of ecosystems beneath ice masses. The evaluation of the SLW microbial community using both 16S rRNA and metagenomic approaches allowed connections to be made between the genetic potential and possible activity of various organisms in the system. However, inconsistencies exist between these datasets, particularly regarding ammonia and methane oxidation, which many be clarified through metatranscriptomic analysis. While this work predominantly focused on the prokaryotic components of SLW and the WIS-GZ, eukaryotic and viral populations were present in the communities and a detailed investigation into their activity and diversity is needed to better understand what role, if any, they play in these ecosystems. Despite their isolation, both SLW and the WIS-GZ are very dynamic systems, experiencing episodic pulses of external input and possible changes in redox conditions. Consequently, temporal and spatial monitoring of these and similar subglacial sites is important to constrain the extent of these environmental fluctuations and evaluate how organisms may respond. Ultimately, further

exploration of subglacial environments is required to truly understand the abundance and diversity of subglacial communities as well as their possible impact on global geochemical cycling.

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APPENDIX A.
SUPPLEMENTAL MATERIAL: CHAPTER 2

Materials and Methods

Microbiological Monitoring During Hot Water Drilling

To minimize the introduction of microbial contamination into SLW during scientific operations, the melted snow and ice used for drilling was sequentially passed through large capacity filters (2.0µm and 0.2µm) and irradiated with UV (185 and 245 nm) to remove and kill cells, respectively. Subsequently, the water was heated to ~90˚C and pumped through ~1 km of drill hose into the borehole (Supplemental Figure 1A). The WISSARD water treatment system was tested extensively and its effectiveness is described in Priscu et al. (2013).

Water was sampled from various ports in the hot water system during drilling and in the borehole via hydrocast (Supplemental Figure 1A) to assess the cell concentration and microbial diversity of the drill water. Once drilling progressed to a depth of ~210 meters below the surface (mbs), 19 L of water was collected from an access port located before the filtration and UV systems and designated sample T1P1 (i.e., time point 1, port 1). Simultaneously, an equal volume from the return pump (designated T1P9) that recirculated water from the borehole to the water treatment system was sampled (Supplemental Figure 1A; Rack, 2016). Drilling was paused at a depth of ~700 mbs to allow visual inspection of the borehole and water sampling. A 10L Niskin bottle was deployed to retrieve a direct sample of the borehole water from a depth of 672 mbs (Supplemental Figure 1A). After drilling resumed and the borehole reached a depth of ~735 mbs, water samples were again collected from ports on the drilling system. Water returning from the borehole was designated T2P9 (time point 2, port 9) and that originating from a port located after the water heater were designated T2P8 (Supplemental Figure 1A). Immediately after collection, cells in the water samples were filter concentrated on 142mm, 0.2 µm Supor membrane filters (Pall Corp.) using a peristaltic pump and sterile tubing.

To disinfect the equipment and instrumentation used during borehole operations, 3% hydrogen peroxide (w/v) was applied to all surfaces with a pressure sprayer and the cleaned materials were staged in sealed polyethylene bags. The filter housing of the Large Volume Water Transfer System (WTS-LV) was cleaned between casts by sequential washes with sterile water, 70% ethanol, and 3% hydrogen peroxide.

Nucleic Acid Extraction, Amplification, and Sequencing

DNA was extracted, amplified, and sequenced as previously described (Christner et al, 2014). RNA was extracted from cells on the filters and reverse transcribed into complementary DNA (cDNA) using the method described by Dieser et al. (2014). For sediment samples, 3 grams (wet weight) were centrifuged at 25k x g for 10 minutes. The supernatant was discarded and the sediment pellet was suspended in 3 mL of TE buffer (1mM EDTA, 10mM Tris; pH 6.3). The slurry was subsequently processed using the same extraction protocol as used for the filters. Procedural blanks that consisted of clean filters were extracted in parallel and served as methodological controls.

Amplification of the V4 region of the 16S rRNA gene was performed in a 50 µl volume using 5 units of AmpliTaq Gold DNA Polymerase LD (Invitrogen) and the following components; 1 x PCR Gold Buffer, 3.5 mM $MgCl_2$, 10 pmol of each primer (515F and 806R; Caporaso et al, 2012), 200 uM dNTPs and 1.5-5 ul of DNA template (0.02-1 ng/pg). The PCR consisted of a 5-8 min initial denaturation at 94 ˚C followed by thirty-five cycles of amplification under the following conditions: 94 ˚C for 45 s, 50 ˚C annealing for 90 s, and elongation at 72˚C for 90 s, with a terminal elongation at 72 ˚C for 10 min. Methodological controls and some of the sediment samples were amplified using up to 40 cycles when no or weak amplification was observed after 35 cycles of amplification. PCR products were evaluated using agarose gel electrophoresis and amplicon concentrations were determined using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies). Several sediment extractions showed poor amplification and were not submitted for sequencing. The amplicons were pooled in equimolar concentrations, and

primers and PCR reagents were removed with the MoBio UltraClean PCR Clean-Up Kit. The pooled amplicons were sequenced on an Illumina MiSeq platform that generated 250 bp paired end reads.

Result

SLW 16S rDNA and rRNA Sequence Diversity and Comparison

After quality filtering removed 28% of the sequence reads, 7,520,040 sequences (65% and 35% of the total rDNA and rRNA sequences, respectively) remained for further analysis. Calculations of sequence coverage (Good's Coverage; Supplemental Table A.1) and rarefaction analysis (data not shown) indicated that the data were sufficient to characterize the dominant microbial community members. The rDNA-derived libraries from the lake water had lower species richness compared to the rRNA based libraries based on the number of observed OTUs and Chao1 richness estimates (Supplemental Table A.1). In general, each of the indices for the rDNA water samples implied that the microbial diversity decreased with filter pore size. This trend was not observed in the rRNA samples, the diversity of which was more uniform across the size fractions. Although sediment richness (observed OTUs and Chao1) was lower in the rRNA versus rDNA data, both richness and estimations of diversity (Inv. Simpson and Shannon) were higher in surficial sediments (0-6cm) than in the deeper horizons (18-36cm; Supplemental Table A.1).

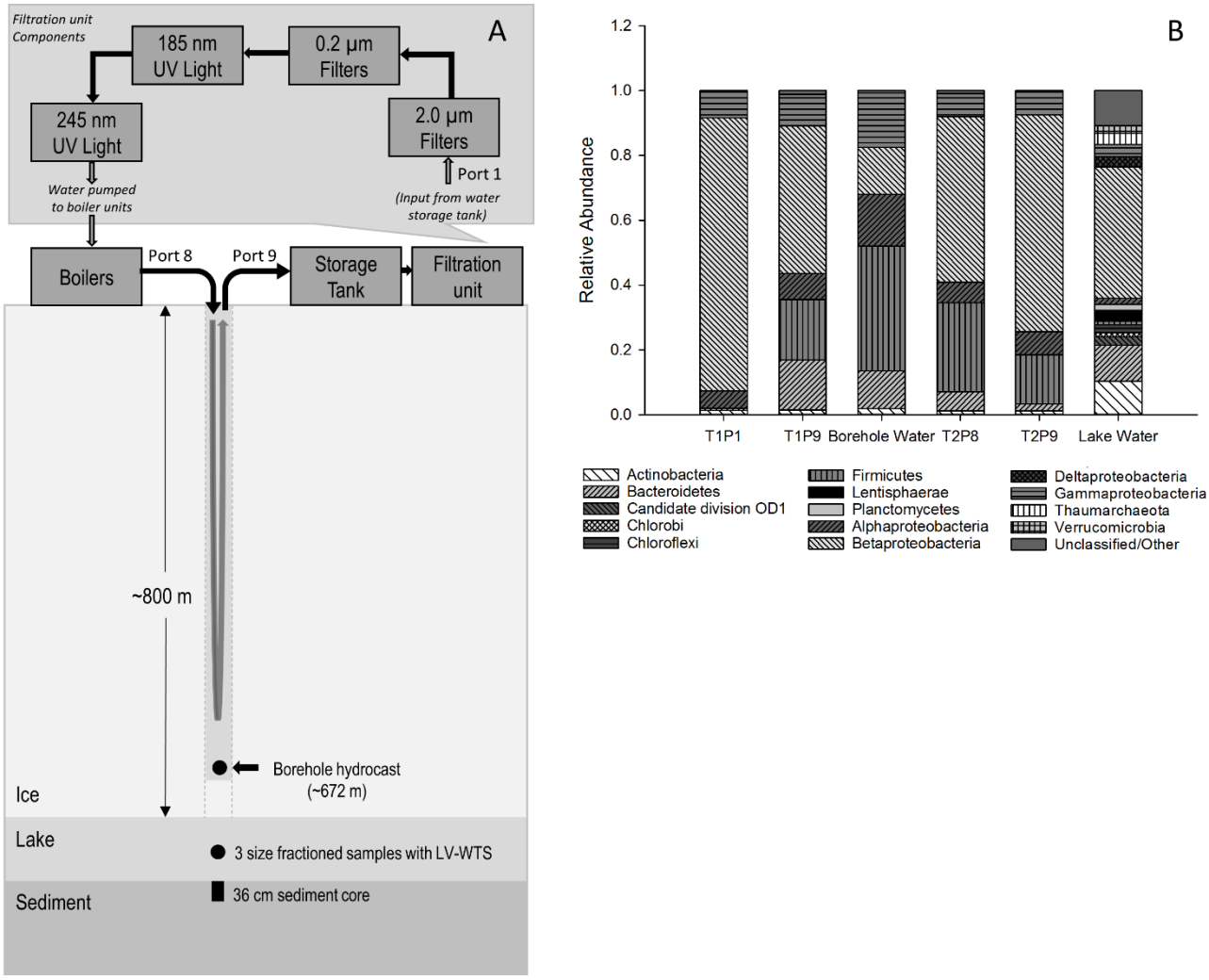
Characterization of Microorganisms in the Drilling Water and Procedural Controls

Comparative analysis of sequences amplified from extracted nucleic acids in water samples that circulated in the drilling system and borehole, extraction blanks, and SLW water and sediments was conducted to assess exchange with the lake and examine the source of phylotypes encountered during scientific operations at SLW. Samples collected at two time points (designated T1 and T2) and locations in the WISSARD water treatment and drilling system (Supplemental Figure 1A) were used to characterize the composition of microorganisms circulating in the drill water, and an additional sample was collected via hydrocast from a depth of 672 mbs in the borehole prior to SLW entry. Betaproteobacteria were the

Supplemental Table A.1. Summary of parameters for 16S rRNA and rRNA gene analysis for SLW separated according to cast, filter size fraction, and sediment depth. Indices were calculated on subsampled data within Mothur and are an average of 1000 iterations. Singletons were included in this analysis.

	Observed OTUs		Inverse Simpson		Shannon Diversity		Chao1		Good's Coverage	
	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA
0-2 cm	98	449	4.8	17.9	2	3.9	185	630	99.6%	98.8%
2-4 cm	256	113	14.1	5.9	3.2	2.2	476	274	98.9%	99.4%
4-6 cm	69	375	1.6	20.8	1	3.8	119	518	99.7%	99.0%
18-20 cm	118	203	4.7	3.7	1.9	2.4	236	423	99.4%	99.2%
34-36 cm	133	144	3.7	1.8	2	1.3	183	302	99.6%	99.3%
Cast 1 10 µm	168	462	9.2	22	2.9	3.9	263	755	99.5%	98.3%
Cast 1 3.0 µm	36	68	4.5	7	1.9	2.3	45	125	99.9%	99.8%
Cast 1 0.2 µm	38	46	2.3	2.3	1.2	1.3	61	75	99.9%	99.8%
Cast 2 3.0 µm	264	306	14.7	9.6	3.2	2.8	418	883	99.0%	98.2%
Cast 2 0.8 µm	229	619	7.6	14.8	2.7	3.5	426	1574	99.0%	96.6%
Cast 2 0.2 µm	80	960	3.4	18.7	1.7	4	156	2754	99.7%	94.3%
Cast 3 3.0 µm	197	425	10.2	9.5	2.8	3	379	1157	99.1%	97.6%
Cast 3 0.8 µm	130	285	5	6.5	2.2	2.5	275	866	99.4%	98.4%
Cast 3 0.2 µm	61	791	2.5	11.7	1.4	3.6	104	2153	99.8%	95.4%

dominant phyla detected in the drilling system (45-84% of the rDNA sequences; Supplemental Figure 1B), being most abundant in water samples obtained prior to filtration and UV treatment (port 1, T1P1; Supplemental Figure 1A), and lowest in the borehole hydrocast sample (Supplemental Figure 1B). The most abundant Betaproteobacterial OTUs in the drilling water were most closely related to species of *Janthinobacterium* (23% of sequence reads), *Delftia* (12%), and *Herbaspirillium* (6%). Members of the *Janthinobacterium* and *Herbaspirillium* were also among the dominant Betaproteobacterial OTUs in the borehole hydrocast sample (2% and 5%, respectively). Although the relative abundance of Alpha- and Gammaproteobacteria in the borehole sample was 16% and 17%, respectively, these classes were found to be consistently lower in the other drilling water samples (5 to 10%, respectively).

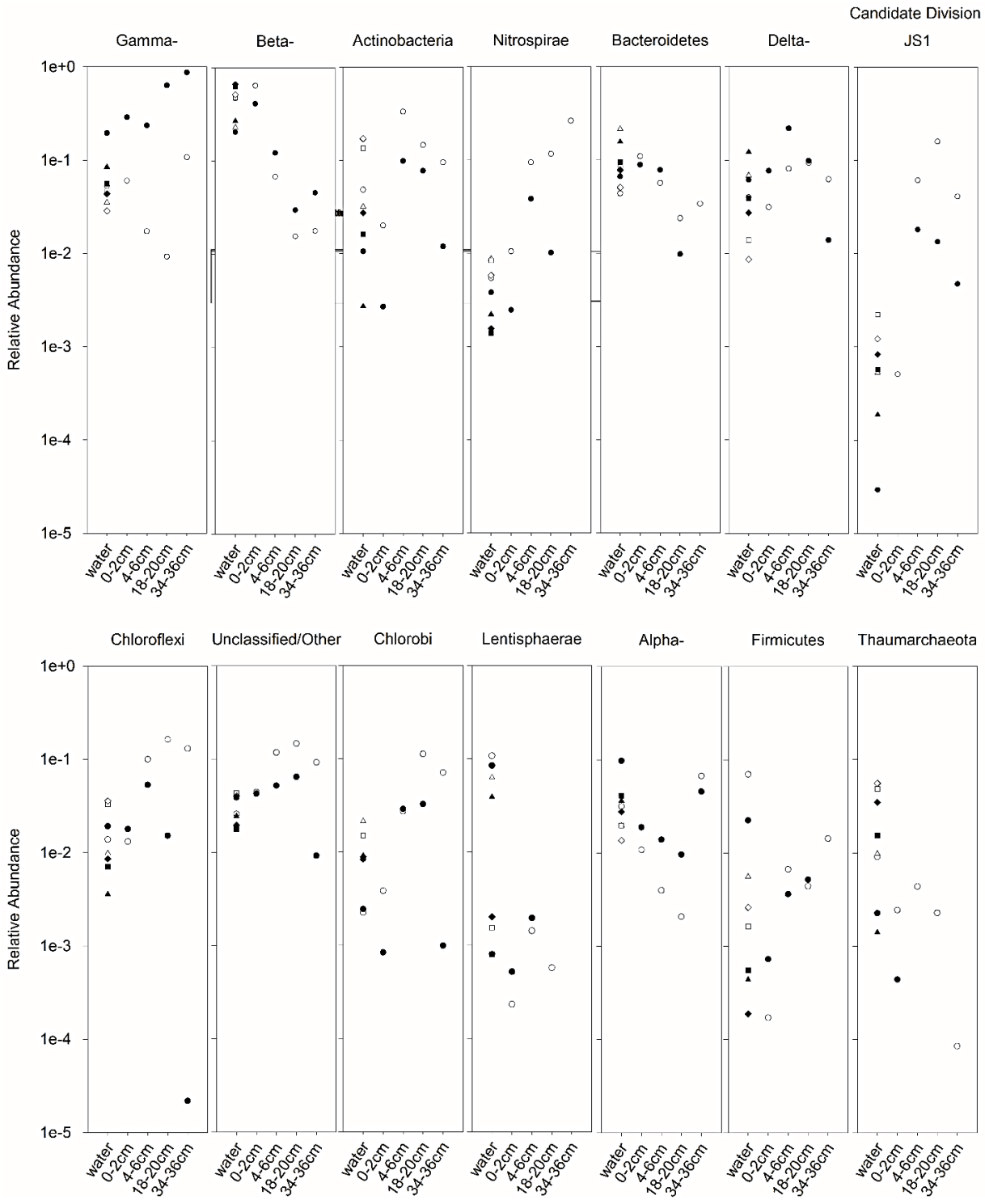


Supplemental Figure A.1. The microorganisms associated with the hot water drill system. (A) Schematic representation of WISSARD's microbiologically-clean hot water drill system, showing the filtration and UV module components, location of sampling ports used, and the SLW access borehole. (B) The relative abundance of the dominant phylum (>1% of sequence reads) in drill water, borehole, and bulk water samples.

OTUs classifying within the Bacteroidetes and Firmicutes were highly abundant in the borehole hydrocast (12 and 39%, respectively) and return well samples (T1P9; 15 and 19% respectively), but were rare in samples from port 1 (Supplemental Figure 1B; 0.7 and 0.06%, respectively). During the second collection time point, the relative abundance of Bacteroidetes phylotypes decreased by ~7-fold in water returning to the surface via the return well pump (T2P9; Supplemental Figure 1B) compared to initial sampling (T1P9). A large portion of the Firmicutes sequences (19%) that were associated with the

drilling and borehole water grouped within a single OTU affiliated with the genus *Tumebacillus,* while *Cloacibacterium* and *Chyseobacterium*-like taxa were the most abundant Bacteroidetes (3.34% and 3%, respectively, of all drill water sequences). OTUs within the genera *Tumebacillus* and *Chryseobacterium,* along with those closely related to *Janthinobacterium* and *Delftia,* were also found in all the lake water samples, and a *Herbasparillium*-like OTU was observed in every rDNA derived library. Combined, these 5 OTUs represented between 37 and 78% of the sequences obtained from the drilling water samples, and between <1% and 27% of the sequence reads from lake water and sediment samples (average of 3%). Despite the presence of several shared OTUs, microbial assemblages in the drilling water were statistically different from those of the lake water and sediment ($p \leq 0.008$), and grouped distinctly in a nonmetric multidimensional scaling (NMDS) analysis (Figure 1).

Procedural blanks were processed using materials and solutions that were identical to those used for extractions of SLW lake water and sediment samples. No PCR amplification was observed in any DNA-based controls that targeted 16S rRNA genes (Christner et al, 2014), and therefore, none were available for sequencing. Weak amplification was observed from blanks processed for the extraction and detection of 16S rRNA molecules, and four of these samples were selected for sequencing and analysis. The blank controls shared 2% of the water column OTUs from the rRNA based libraries and 14% of those from the sediments. Less than 3% of the OTUs in the blanks were shared with those in rDNA derived libraries and were considered likely contaminants that originated from procedures associated with nucleic acid extraction, amplification, and/or sequencing. Hence, they were not considered further in the SLW microbial community description. The majority of OTUs from the rRNA controls classified as Gammaproteobacteria (average of 57%), while 21% was comprised of Betaproteobacteria, and 11% of Firmicutes. A large portion of the Gammaproteobacterial reads classified within three OTUs closely related to species of *Halomonas* (15% of the rRNA control reads), *Escherichia* (10%), and *Pseudomonas* (5%).



Supplemental Figure A.2**.** The relative abundance of the 14 most abundant phyla in the SLW water column and sediments. Data from size fractions in the water column and sediment depths are indicated. White and black symbols denote 16S rDNA and rRNA sequence abundances, respectively. Within the water the 10um size fraction is denoted by circles, the 3um by triangle, the 0.8um by square, and the 0.2um by diamond.

Supplemental Table A.2. Mothur based taxonomic classification of abundant OTUs shown in Figure 2.2 using the SEED database. Bootstrap confidence values are given in parentheses.

OTU	Phylum	Class	Order	Family	Genus
Otu000038	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	unclassified(100)
Otu000042	Planctomycetes(100)	Planctomycetacia(100)	Brocadiales(100)	Brocadiaceae(100)	unclassified(100)
Otu000051	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	unclassified(100)
Otu000058	Proteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000064	Proteobacteria(100)	Betaproteobacteria(100)	Hydrogenophilales(100)	Hydrogenophilaceae(100)	Thiobacillus(100)
Otu000073	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	Candidatus_Nitrotoga(100)
Otu000074	Thaumarchaeota(100)	Marine_Group_I(100)	Unknown_Order(100)	Unknown_Family(100)	Candidatus_Nitrosoarchaeum(100)
Otu000076	Lentisphaerae(100)	WCHB1-41(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000078	Nitrospirae(100)	Nitrospira(100)	Nitrospirales(100)	Nitrospiraceae(100)	unclassified(100)
Otu000093	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	KD3-93(100)	unclassified(100)
Otu000098	Actinobacteria(100)	Actinobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000112	Proteobacteria(100)	Gammaproteobacteria(100)	Methylococcales(100)	Methylococcaceae(100)	Methylobacter(100)
Otu000118	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	unclassified(100)
Otu000139	Actinobacteria(100)	Acidimicrobiia(100)	Acidimicrobiales(100)	Acidimicrobiaceae(100)	CL500-29_marine_group(100)
Otu000167	Chlorobi(100)	Chlorobia(100)	Chlorobiales(100)	OPB56(100)	unclassified(100)
Otu000186	Chloroflexi(100)	SL56_marine_group(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000197	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacterales(100)	Syntrophaceae(100)	Syntrophus(100)
Otu000217	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Nitrosomonadaceae(100)	Nitrosospira(100)
Otu000223	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000230	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000340	Lentisphaerae(100)	PBS-III-20(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000370	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacterales(100)	Syntrophaceae(100)	Syntrophus(100)
Otu000409	Proteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000418	Actinobacteria(100)	Actinobacteria(100)	Frankiales(100)	Sporichthyaceae(100)	hgcI_clade(100)
Otu000432	Proteobacteria(100)	Betaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000528	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Polaromonas(100)

Supplemental Table A.2 (Continued)

OTU	Phylum	Class	Order	Family	Genus
Otu000564	Chlorobi(100)	Ignavibacteria(100)	Ignavibacteriales(100)	Ignavibacteriaceae(100)	Ignavibacterium(100)
Otu000575	Chloroflexi(100)	Anaerolineae(100)	Anaerolineales(100)	Anaerolineaceae(100)	unclassified(100)
Otu000586	Bacteroidetes(100)	Cytophagia(100)	Cytophagales(100)	Cytophagaceae(100)	unclassified(100)
Otu000690	Proteobacteria(100)	Gammaproteobacteria(100)	Chromatiales(100)	Ectothiorhodospiraceae(100)	Acidiferrobacter(100)
Otu000916	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacterales(100)	Syntrophaceae(100)	Syntrophus(100)
Otu001193	Proteobacteria(100)	Gammaproteobacteria(100)	Pseudomonadales(100)	Moraxellaceae(100)	Acinetobacter(100)
Otu002036	Proteobacteria(100)	Gammaproteobacteria(100)	Chromatiales(100)	Ectothiorhodospiraceae(100)	Acidiferrobacter(100)
Otu002498	Proteobacteria(100)	Deltaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu002673	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Albidiferax(100)
Otu003072	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Albidiferax(100)
Otu003109	Actinobacteria(100)	OPB41(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu003629	Proteobacteria(100)	Betaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu003751	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	PHOS-HE51(100)	unclassified(100)
Otu003916	Candidate_division_JS1(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu009900	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	PHOS-HE51(100)	unclassified(100)
Otu014447	Chloroflexi(100)	Anaerolineae(100)	Anaerolineales(100)	Anaerolineaceae(100)	unclassified(100)
Otu022091	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	Candidatus_Nitrotoga(100)

Supplemental Table A.3. Mothur based taxonomic classification of OTUs with the highest rRNA:rDNA ration shown in Figure 2.3 using the SEED database. Bootstrap confidence values are given in parentheses.

OTU	Phylum	Class	Order	Family	Genus
Otu000112	Proteobacteria(100)	Gammaproteobacteria(100)	Methylococcales(100)	Methylococcaceae(100)	Methylobacter(100)
Otu000221	Proteobacteria(100)	Alphaproteobacteria(100)	Sphingomonadales(100)	unclassified(100)	unclassified(100)
Otu000279	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	Sideroxydans(100)
Otu000307	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacterales(100)	Syntrophaceae(100)	Syntrophus(100)
Otu000370	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacterales(100)	Syntrophaceae(100)	Syntrophus(100)
Otu000380	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	unclassified(100)
Otu000583	Planctomycetes(100)	Planctomycetacia(100)	Brocadiales(100)	Brocadiaceae(100)	unclassified(100)
Otu000609	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfarculales(100)	Desulfarculaceae(100)	unclassified(100)
Otu000690	Proteobacteria(100)	Gammaproteobacteria(100)	Chromatiales(100)	Ectothiorhodospiraceae(100)	Acidiferrobacter(100)
Otu000733	Proteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu001065	Proteobacteria(100)	Alphaproteobacteria(100)	Sphingomonadales(100)	Erythrobacteraceae(100)	Erythrobacter(100)
Otu001193	Proteobacteria(100)	Gammaproteobacteria(100)	Pseudomonadales(100)	Moraxellaceae(100)	Acinetobacter(100)
Otu001312	Chlorobi(100)	Chlorobia(100)	Chlorobiales(100)	OPB56(100)	unclassified(100)
Otu002195	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	KD3-93(100)	unclassified(100)
Otu002498	Proteobacteria(100)	Deltaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu002533	Proteobacteria(100)	Betaproteobacteria(100)	Nitrosomonadales(100)	Gallionellaceae(100)	unclassified(100)
Otu002673	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Albidiferax(100)
Otu005695	Chloroflexi(100)	Anaerolineae(100)	Anaerolineales(100)	Anaerolineaceae(100)	unclassified(100)
Otu006794	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Tabrizicola(99)
Otu008210	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Burkholderiaceae(100)	unclassified(100)
Otu010260	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacterales(100)	Syntrophaceae(100)	unclassified(100)
Otu013742	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfobacterales(100)	Desulfobacteraceae(100)	unclassified(100)
Otu015663	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu016841	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu022106	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu024095	Armatimonadetes(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu025293	Proteobacteria(100)	Deltaproteobacteria(100)	Myxococcales(100)	Polyangiaceae(100)	Sorangium(100)
Otu034306	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)

APPENDIX B.
SUPPLEMENTAL MATERIAL: CHAPTER 3

Supplemental Table B.1: List of KEGG Orthologs involved in carbon, nitrogen, and sulfur cycling that were evaluated in this study.

Carbon

Step	KEGG	Gene
Aerobic C fixation	K00855	phosphoribulokinase
	K01602	ribulose-bisphosphate carboxylase small (*cbbS*)
	K01601	ribulose-bisphosphate carboxylase large (*cbbL*)
Aerobic respiration	K02256	cytochrome c oxidase subunit I (coxI)
	K02262	cytochrome c oxidase subunit III (coxIII)
	K02274	cytochrome c oxidase subunit I (coxA)
	K02276	cytochrome c oxidase subunit III (coxC)
Aerobic methane oxidation	K08684	methane monooxygenase
	K01944*	methane/ammonia monooxygenase subunit A
	K10945*	methane/ammonia monooxygenase subunit B
	K10946*	methane/ammonia monooxygenase subunit C
Anaerobic C fixation	K00174	2-oxoglutarate:ferredoxin oxidoreductase subunit alpha
	K00175	2-oxoglutarate:ferredoxin oxidoreductase subunit beta
	K00244	frdA; fumarate reductase flavoprotein subunit
	K01648	ATP citrate lyase
	K00194	CO dehydrogenase subunit delta
	K00197	CO dehydrogenase subunit gamma
	K14534	4-hydroxybutyryl-CoA dehydratase
Fermentation	K00016	L-lactate dehydrogenase
	K00169	pyruvate:ferredoxin oxidoreductase alpha (*porA*)
	K00170	pyruvate:ferredoxin oxidoreductase beta (*porB*)
CO oxidation	K03518	CO dehydrogenase small subunit (coxS)
	K03519	cutM, coxM; carbon-monoxide dehydrogenase medium subunit
	K03520	cutL, coxL; carbon-monoxide dehydrogenase large subunit
Methanogenesis	K00400	coenzyme M methyl reductase beta subunit (mcrB)
	K00401	methyl coenzyme M reductase system, component A2

Nitrogen

Step	KEGG	Gene
DNRA	K03385	formate-dependent nitrite reductase periplasmic cytochrome c552 (nrfA)
	K05904	cytochrome c nitrite reductase (nrfA)
anammox	K10535	hydroxylamine oxidoreductase/hydrazine oxidoreducatse (hao/hzo)
Denitrification	K00376	nitrous oxide reductase (nosZ)
	K02305	nitric-oxide reductase (norC)
	K04561	nitric-oxide reductase (norB)

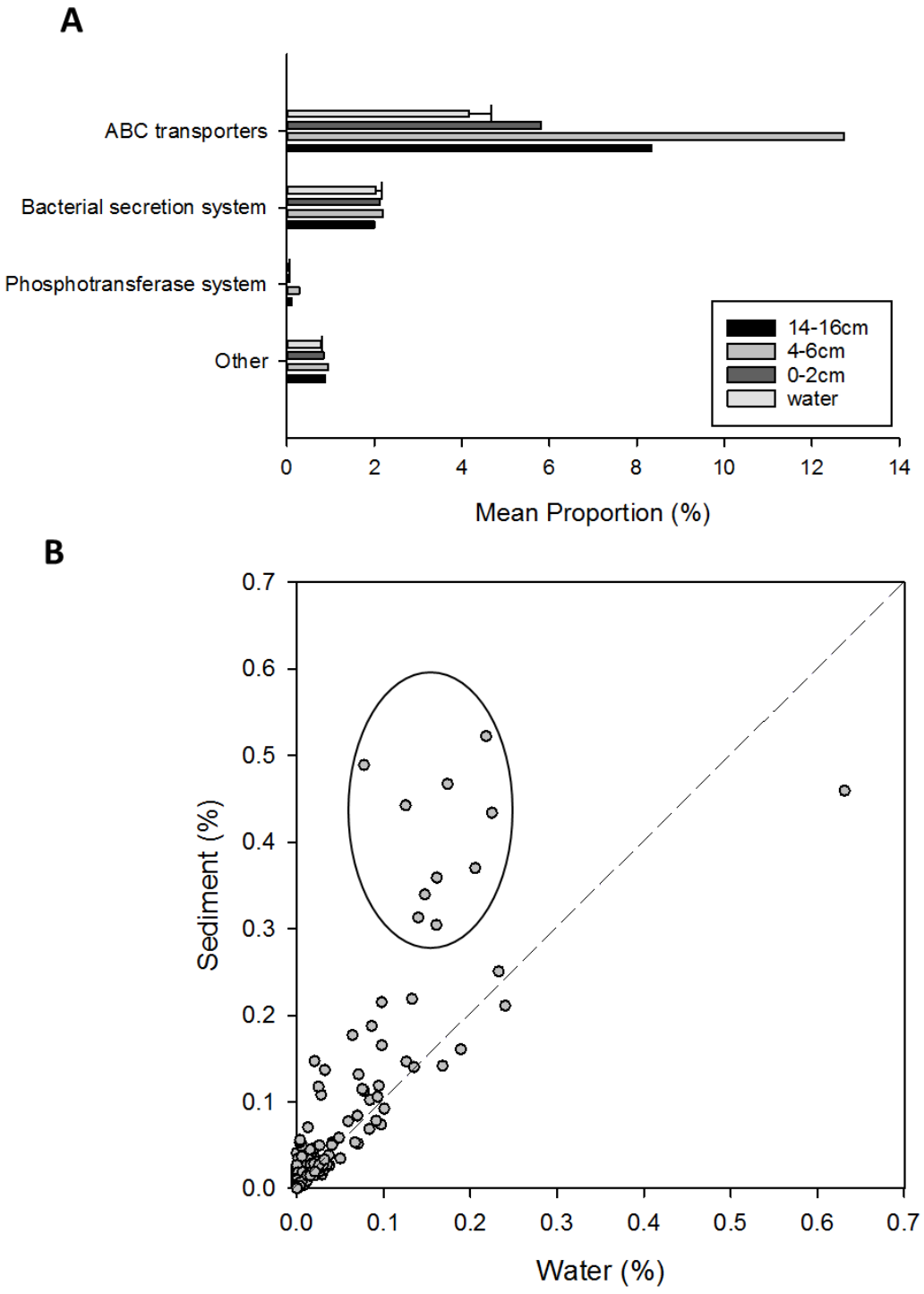
Table B.1 (Continued)

Step	KEGG	Gene
Dissimilatory nitrate reduction	K02567	periplasmic nitrate reductase (napA)
	K02568	cytochrome c-type protein (napB)
Nitrification	K10944*	methane/ammonia monooxygenase subunit A
	K10945*	methane/ammonia monooxygenase subunit B
	K10946*	methane/ammonia monooxygenase subunit C
Nitrogen assimilation	K00265	glutamate synthase (NADPH/NADH) large chain (gltB)
	K00284	glutamate synthase (ferredoxin-dependent) (gltS)
	K01915	glutamine synthetase (glnA)
	K00372	assimilartory nitrate reductase (nasA)
	K00360	assimilartory nitrate reductase (nasB)
	K00367	assimilartory nitrate reductase (narB)
Nitrogen fixation	K00531	nitrogenase
	K02586	nitrogenase molybdenum-iron protein alpha chain (nifD)
	K02588	nitrogenase iron protein (nifH)
	K02591	nitrogenase molybdenum-iron protein beta chain (nifK)
Nitrogen mineralization	K00260	glutamate dehydrogenase
	K00261	glutamate dehydrogenase
	K00262	glutamate dehydrogenase
Sulfur		
Step	KEGG	Gene
assimilatory sulfate reduction	K00860	adenylylsulfate kinase (cysC)
	K00956	sulfate adenylyltransferase subunit 1 (cysN)
	K00957	sulfate adenylyltransferase subunit 2 (cysD)
Dissimilatory sulfate reduction and sulfide oxidation	K00394	adenylylsulfate reductase subunit A (aprA)
	K00385	adenylylsulfate reductase subunit B (aprB)
	K11180	sulfite reductase (dsrA)
	K11181	sulfite reductase (dsrB)
	K17224	soxB
sulfur mineralization	K00456	cysteine dioxygenase
	K01011	3-mercaptopyruvate sulfurtransferase

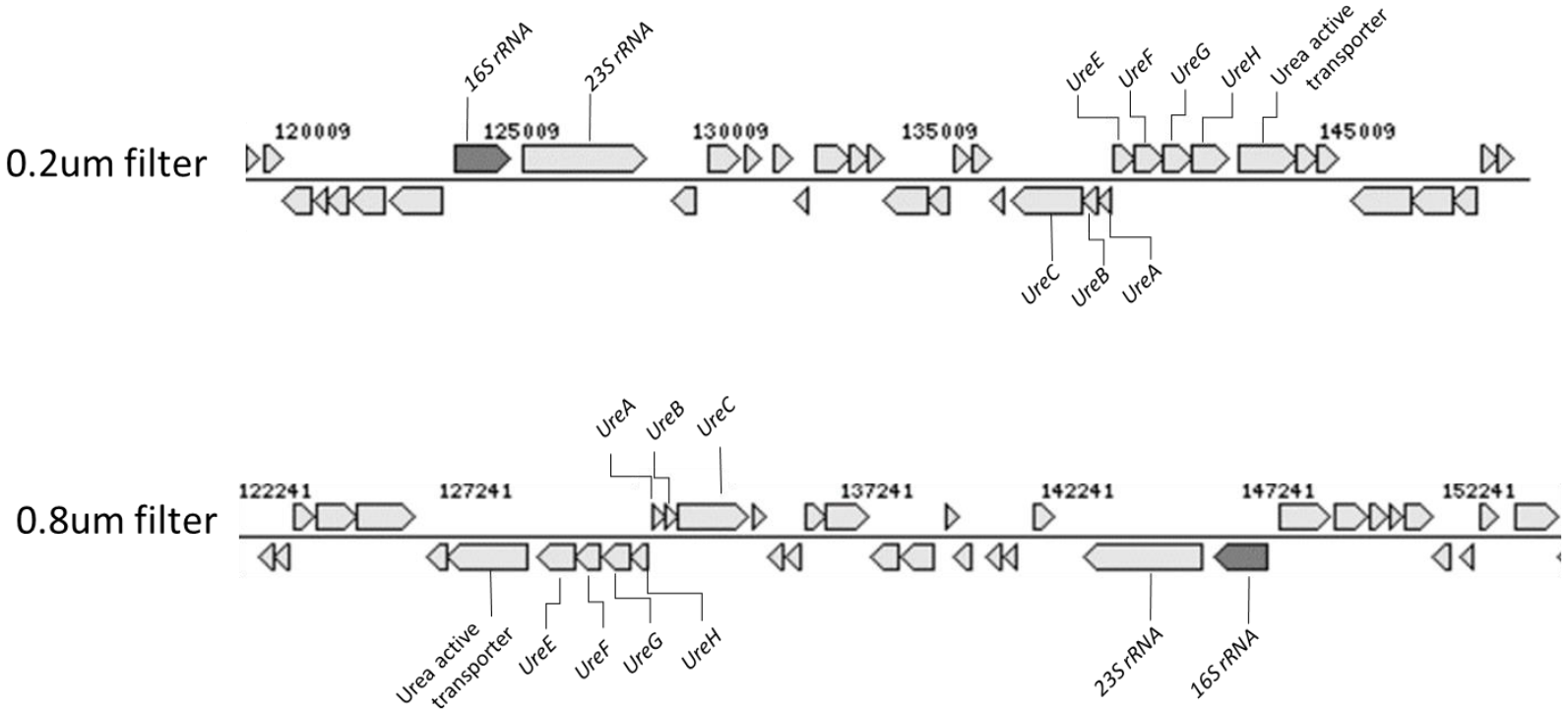
*Marker genes K10944-K10946 were assigned to either methane or ammonia oxidative processes based on taxonomic affiliation.

Supplemental Table B.2: Results of assembly of metagenomic reads using IDBA-UD.

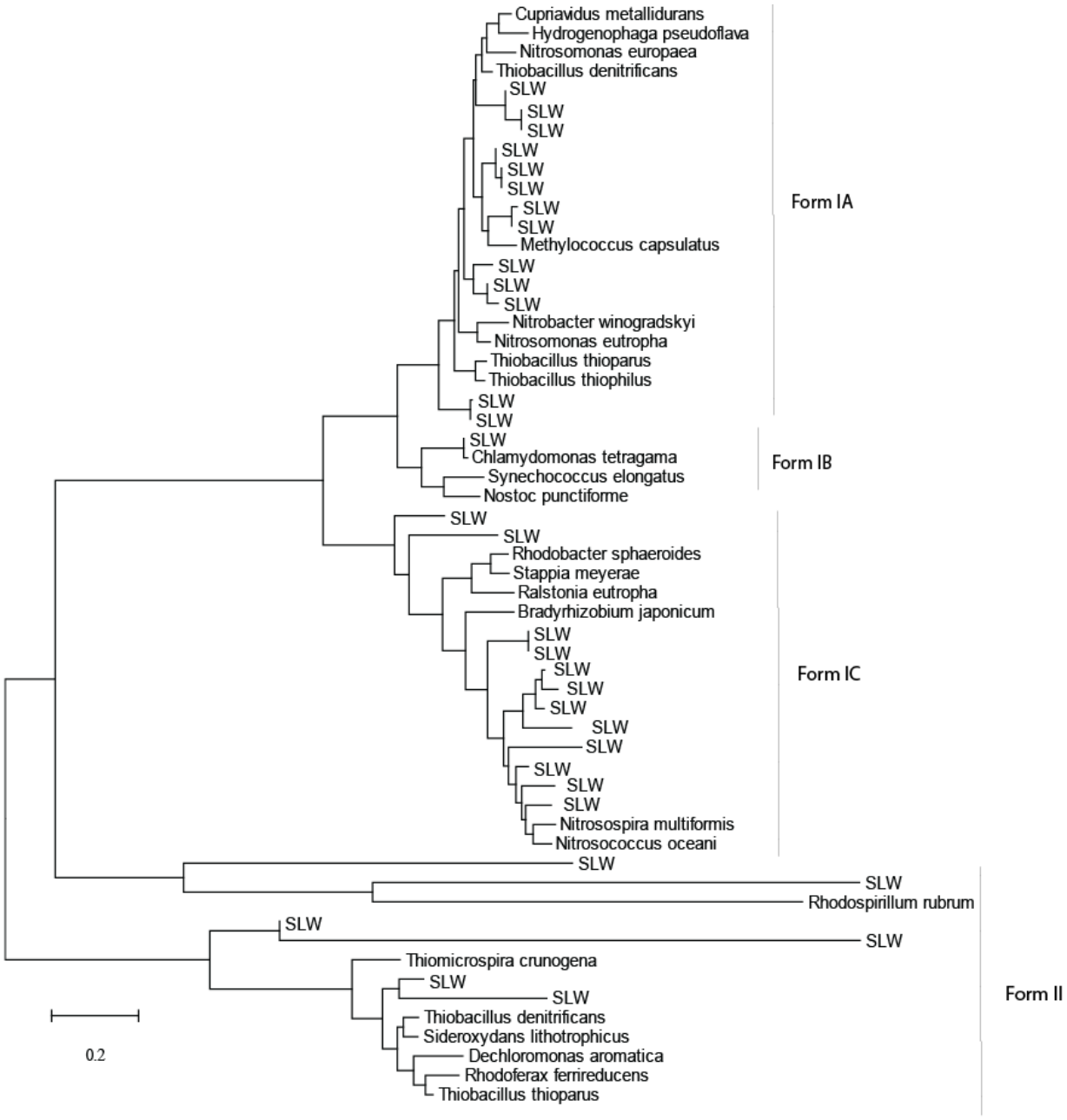
		Percent Aligned Reads	Number of Scaffolds	N50	Maximum Length	Mean
Water	0.2um	27.2%	10,181	2,354	260,299	1,701
	0.8um	27.5%	17096	2,364	247,669	1,668
	3.0um	18.6%	15,764	1,315	40,649	1,600
Sediment	0-2cm	13.3%	12,125	1,049	14,459	1,071
	4-6cm	40.7%	21,313	3,499	105,855	2,119
	14-16cm	24.2%	21,029	1,584	29,562	1,391



Supplemental Figure B.1. (A) The distribution of genes associated with transport and (B) their relative abundance in the water column as compared to the sediments. Circle denotes genes involved in peptide/dipeptide and branched-chain amino acid transport.



Supplemental Figure B.2. Portions of assembled genomic fragments from the SLW water column metagenomic dataset (0.2um and 0.8 um samples) that contain archaeal 16S rRNA genes and genes involved in the transport and utilization of urea.



Supplemental Figure B.3. Phylogenetic analysis of *cbbL* and *cbbM* amino acid sequences using maximum likelihood method. Branch length represent the number of substitutions per site.

APPENDIX C.
SUPPLEMENTAL MATERIAL: CHAPTER 4

Supplemental Table C.1: Summary of parameters for 16S rRNA and rRNA gene analysis for WIS-GZ separated according to cast and filter size fraction and sediment depth. Indices were calculated within Mothur on subsampled data and are an average of 1000 iterations. Singletons included.

	OTUs		Chao1		Inv. Simpson		Shannon		Coverage	
	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA	rDNA	rRNA
Cast 1 3.0um	2349	235	6652.9	400.0	64.1	11.9	5.2	3.1	94.0%	99.6%
Cast 1 0.8um	1421	1067	5006.5	3457.8	42.9	14.3	4.5	3.7	96.2%	97.3%
Cast 1 0.2um	1343	742	3989.9	2192.8	7.6	8.2	3.4	3.0	96.5%	98.2%
Cast 2 3.0um	1632	1098	3828.9	3127.3	46.4	31.9	4.8	4.3	96.3%	97.4%
Cast 2 0.8um	1487	986	4769.9	3019.6	46.4	13.0	4.6	3.6	96.2%	97.5%
Cast 2 0.2um	946	667	2835.9	2171.1	3.6	6.9	2.5	2.7	97.6%	98.3%
Cast 3 3.0um	2374	710	3859.6	1733.9	156.1	22.0	6.0	3.9	95.8%	98.5%
Cast 3 0.8um	1921	608	5633.0	1750.4	59.0	10.5	5.0	3.2	95.2%	98.6%
Cast 3 0.2um	1180	479	3626.8	1374.1	6.9	6.2	3.1	2.6	96.9%	98.9%
Cast 4 3.0um	1674	1256	3857.8	3521.2	61.4	33.6	5.0	4.4	96.2%	96.9%
Cast 4 0.8um	1323	1005	4284.9	2634.2	38.8	15.5	4.5	3.7	96.6%	97.6%
Cast 4 0.2um	563	646	1559.9	1950.7	7.0	7.7	2.9	2.9	98.7%	98.4%
0-2cm	1343	975	1832.8	1246.0	47.0	58.9	5.1	5.3	98.4%	99.2%
2-4cm	1744	1016	2648.2	1975.4	71.3	33.2	5.4	4.3	97.4%	97.9%
4-6cm	2132	679	3825.4	942.1	55.2	42.0	5.2	4.9	95.8%	99.4%
6-8cm	2050	1182	3608.4	1688.4	45.7	29.8	5.0	4.4	96.0%	98.2%
8-10cm	1353	1097	2186.3	1974.2	18.9	12.6	4.2	3.6	97.5%	97.8%
10-12cm	596	513	843.4	807.1	10.7	10.4	3.6	3.8	99.3%	99.4%

Supplemental Table C.2: Summary of parameters for SLW, WIS-GZ, and MIS samples. Indices were calculated within Mothur on subsampled data and are an average of 1000 iterations. Singletons included.

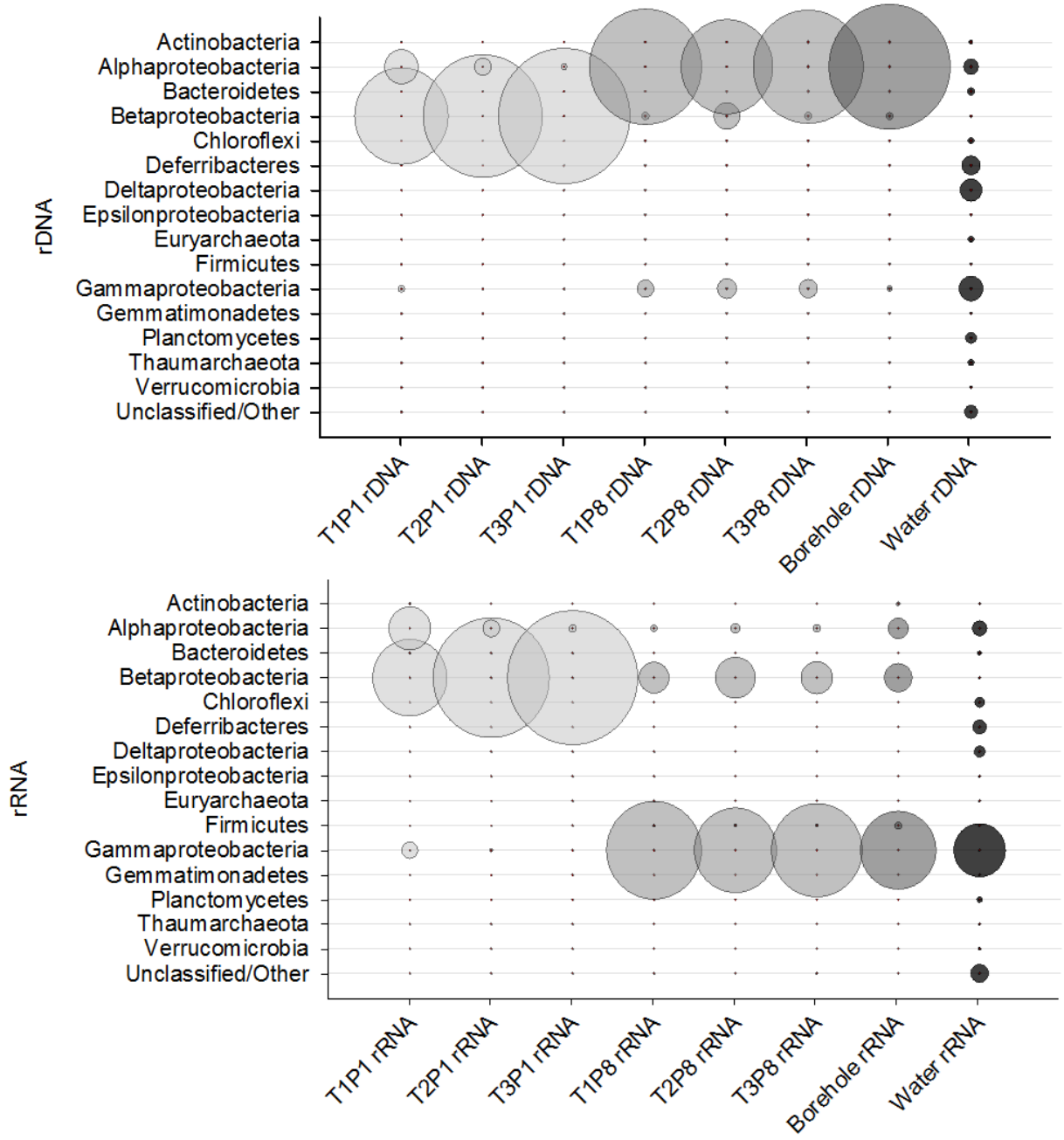
	OTUs	Chao1	Inv. Simpson	Shannon	Coverage
SLW Water	24770	39165.8	197.6	6.6	99.4%
WIS-GZ Water	69960	88413.4	382.9	8.2	98.5%
MIS Water	38975	82337.5	120.2	6.2	98.6%
SLW Sediment	12142	20294.7	83.1	5.5	99.7%
WIS-GZ Sediment	17085	23539.5	87.8	6.2	99.6%

Supplemental Table C.3. Mothur based taxonomic classification of OTUs with the highest rRNA:rDNA ration shown in Figure 2.3 using the SEED database. Bootstrap confidence values are given in parentheses.

OTU	Phylum	Class	Order	Family	Genus
Otu000004	Proteobacteria(100)	Deltaproteobacteria(100)	SAR324_clade(100)	unclassified(100)	unclassified(100)
Otu000033	Proteobacteria(100)	Gammaproteobacteria(100)	Incertae_Sedis(100)	Unknown_Family(100)	Thiohalophilus(100)
Otu000039	Chloroflexi(100)	SAR202_clade(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000064	Proteobacteria(100)	Betaproteobacteria(100)	Hydrogenophilales(100)	Hydrogenophilaceae(100)	Thiobacillus(100)
Otu000078	Nitrospirae(100)	Nitrospira(100)	Nitrospirales(100)	Nitrospiraceae(100)	unclassified(100)
Otu000148	Actinobacteria(100)	Acidimicrobiia(100)	Acidimicrobiales(100)	OM1_clade(100)	unclassified(100)
Otu000152	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000172	Proteobacteria(100)	Deltaproteobacteria(100)	SAR324_clade(100)	unclassified(100)	unclassified(100)
Otu000178	Deferribacteres(100)	Deferribacteres(100)	Deferribacterales(100)	SAR406_clade(100)	unclassified(100)
Otu000193	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000196	Proteobacteria(100)	Alphaproteobacteria(100)	Rhizobiales(100)	Phyllobacteriaceae(100)	Cohaesibacter(100)
Otu000456	Proteobacteria(100)	Gammaproteobacteria(100)	Incertae_Sedis(100)	Unknown_Family(100)	Thiohalophilus(100)
Otu000547	Actinobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000624	Proteobacteria(100)	Alphaproteobacteria(100)	Rickettsiales(100)	TK34(100)	unclassified(100)
Otu000627	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000628	Proteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu000670	Actinobacteria(100)	Acidimicrobiia(100)	Acidimicrobiales(100)	OM1_clade(100)	unclassified(100)
Otu000830	Thaumarchaeota(100)	Marine_Group_I(100)	Unknown_Order(100)	Unknown_Family(100)	Candidatus_Nitrosopumilus(100)
Otu000901	Proteobacteria(100)	Gammaproteobacteria(100)	Chromatiales(100)	Ectothiorhodospiraceae(100)	unclassified(100)
Otu000924	Proteobacteria(100)	Deltaproteobacteria(100)	SAR324_clade(100)	unclassified(100)	unclassified(100)
Otu001014	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	JTB255_marine_benthic(100)	unclassified(100)
Otu001134	Proteobacteria(100)	Gammaproteobacteria(100)	Incertae_Sedis(100)	Unknown_Family(100)	Thiohalophilus(100)
Otu001162	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu001211	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu001257	Proteobacteria(100)	Alphaproteobacteria(100)	Rickettsiales(100)	TK34(100)	unclassified(100)
Otu001272	Gemmatimonadetes(100)	Gemmatimonadetes(100)	PAUC43f_marine_benthic(100)	unclassified(100)	unclassified(100)
Otu001297	Proteobacteria(100)	JTB23(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu001365	Proteobacteria(100)	JTB23(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu002036	Proteobacteria(100)	Gammaproteobacteria(100)	Chromatiales(100)	Ectothiorhodospiraceae(100)	Acidiferrobacter(100)
Otu002040	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	JTB255_marine_benthic(100)	unclassified(100)
Otu003830	Thaumarchaeota(100)	Marine_Group_I(100)	Unknown_Order(100)	Unknown_Family(100)	Candidatus_Nitrosopumilus(100)
Otu004778	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu005947	Proteobacteria(100)	Deltaproteobacteria(100)	SAR324_clade(100)	unclassified(100)	unclassified(100)
Otu006234	Proteobacteria(100)	Gammaproteobacteria(100)	Incertae_Sedis(100)	Unknown_Family(100)	Thiohalophilus(100)
Otu007259	Proteobacteria(100)	Deltaproteobacteria(100)	43F-1404R(100)	unclassified(100)	unclassified(100)
Otu008881	Proteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	unclassified(100)
Otu009461	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	unclassified(100)

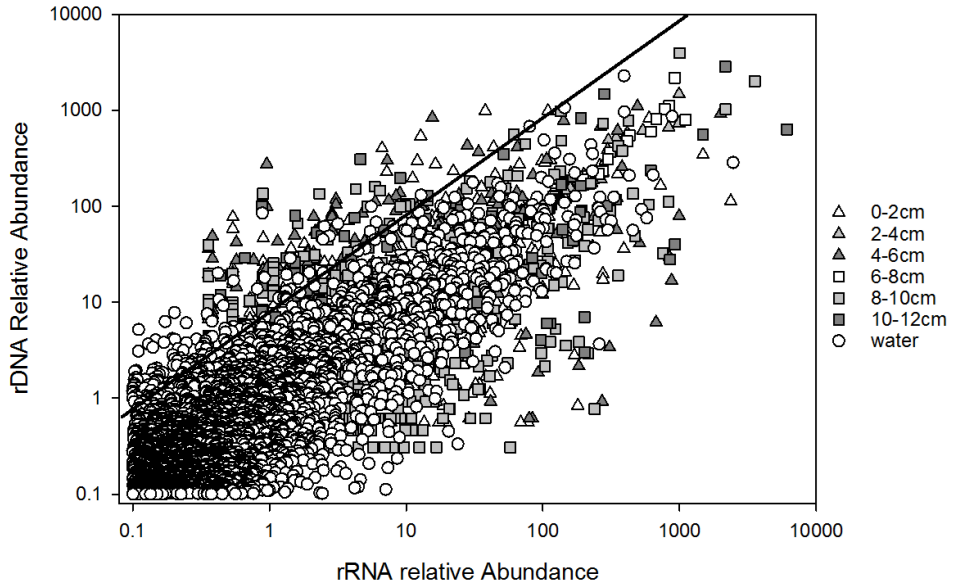
Supplemental Table B.3. (Continued)

OTU	Phylum	Class	Order	Family	Genus
Otu010882	Actinobacteria(100)	Acidimicrobiia(100)	Acidimicrobiales(100)	OM1_clade(100)	unclassified(100)
Otu019618	Proteobacteria(100)	Deltaproteobacteria(100)	DTB120(100)	unclassified(100)	unclassified(100)
Otu028737	Proteobacteria(100)	Gammaproteobacteria(100)	Order_Incertae_Sedis(100)	Family_Incertae_Sedis(100)	Marinicella(100)



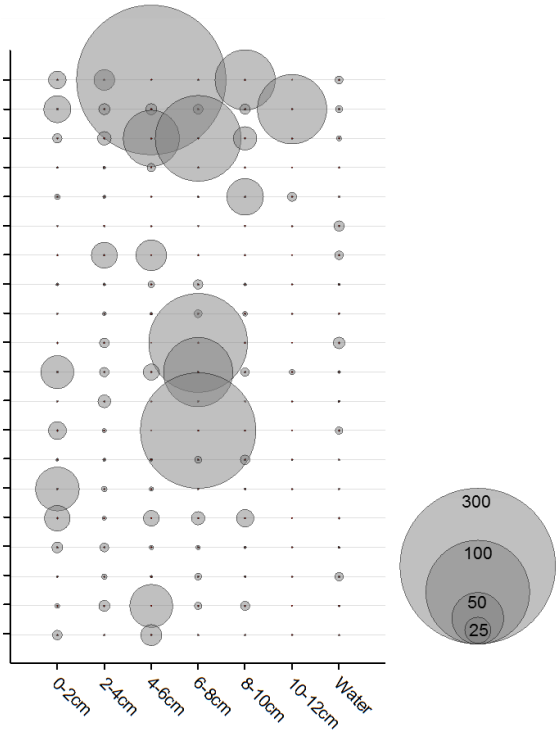
Supplemental Figure C.1: The relative abundance of the dominant phylum (>1% of sequence reads) in drill water, borehole, and bulk water column samples based on rDNA (Top) and rRNA (Bottom) derived libraries.

A

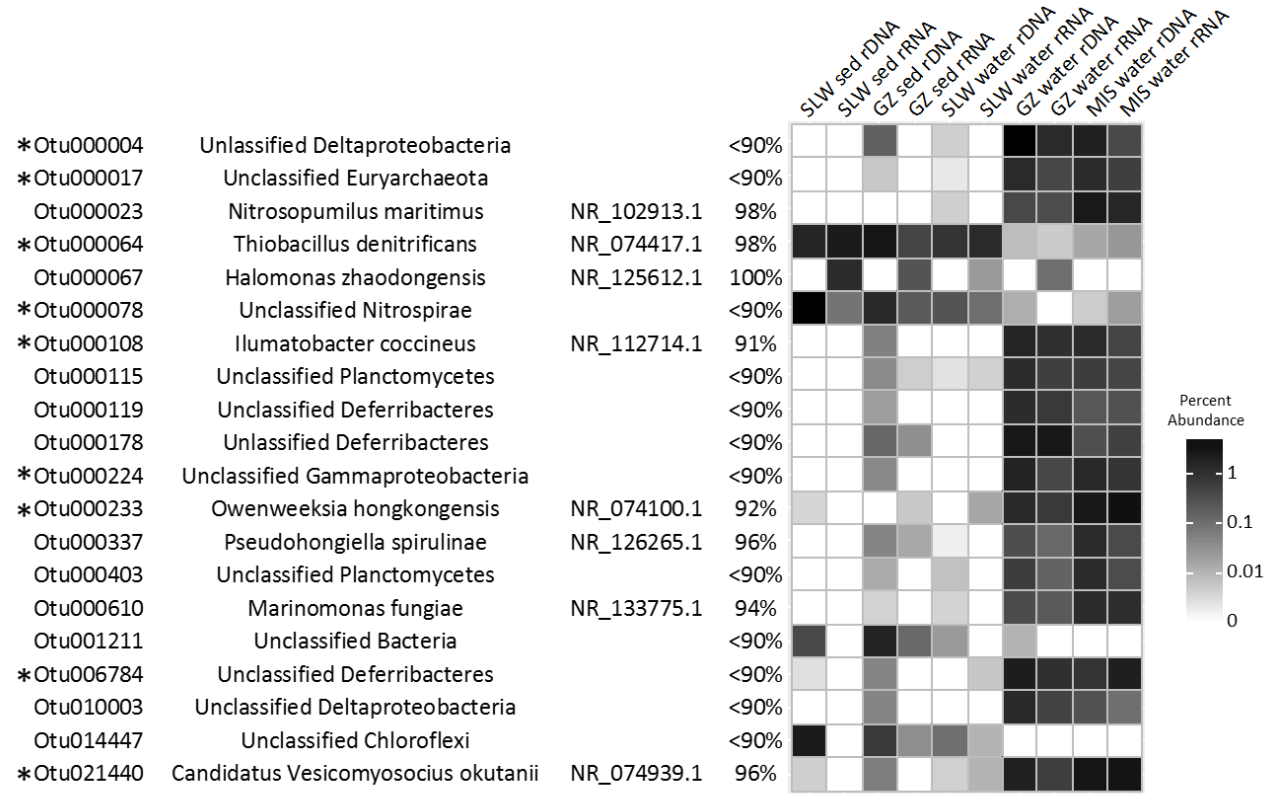


B

Otu000152	Thiohalophilus thiocyanatoxydans	NR_043875.1	94%
Otu000172	Unclassified Deltaproteobacteria		<90%
Otu000193	Melitea salexigens	NR_118016.1	95%
Otu000443	Lacibacterium aquatile	NR_125556.1	91%
Otu000513	Unclassified Actinobacteria		<90%
Otu000525	Thiohalophilus thiocyanatoxydans	NR_043875.1	95%
Otu000591	Thiohalophilus thiocyanatoxydans	NR_043875.1	96%
Otu000628	Thioprofundum hispidum	NR_112620.1	94%
Otu000680	Magnetospira thiophila	NR_116475.1	93%
Otu000704	Kofleria flava	NR_041981.1	92%
Otu000924	Unclassified Deltaproteobacteria		<90%
Otu001016	Unclassified Deltaproteobacteria		<90%
Otu001221	Nesiotobacter exalbescens	NR_041917.1	96%
Otu001257	Azospirillum amazonense	NR_104981.1	94%
Otu001470	Azospirillum halopraeferens	NR_117479.1	92%
Otu002345	Unclassifed Deltaproteobacteria		<90%
Otu003561	Puniceispirillum marinum	CP001751	92%
Otu006234	Thiohalophilus thiocyanatoxydans	NR_043875.1	95%
Otu007419	Sandaracinus amylolyticus	NR_118001.1	91%
Otu008354	Rhodovulum sulfidophilum	NR_115746.1	92%



Supplemental Figure C.2A: The relative abundance of individual OTUs in rRNA versus the rDNA sequence datasets. The black line denotes those OTUs that represent major outliers (>7.6). 2B: List of WIS-GZ OTUs with 16S rRNA:rDNA ratios representative of major outliers within the dataset (≥7.6), identified by their nearest taxonomic neighbor.



*Otu000004	Unlassified Deltaproteobacteria		<90%
*Otu000017	Unclassified Euryarchaeota		<90%
Otu000023	Nitrosopumilus maritimus	NR_102913.1	98%
*Otu000064	Thiobacillus denitrificans	NR_074417.1	98%
Otu000067	Halomonas zhaodongensis	NR_125612.1	100%
*Otu000078	Unclassified Nitrospirae		<90%
*Otu000108	Ilumatobacter coccineus	NR_112714.1	91%
Otu000115	Unclassified Planctomycetes		<90%
Otu000119	Unclassified Deferribacteres		<90%
Otu000178	Unlassified Deferribacteres		<90%
*Otu000224	Unclassified Gammaproteobacteria		<90%
*Otu000233	Owenweeksia hongkongensis	NR_074100.1	92%
Otu000337	Pseudohongiella spirulinae	NR_126265.1	96%
Otu000403	Unclassified Planctomycetes		<90%
Otu000610	Marinomonas fungiae	NR_133775.1	94%
Otu001211	Unclassified Bacteria		<90%
*Otu006784	Unclassified Deferribacteres		<90%
Otu010003	Unclassified Deltaproteobacteria		<90%
Otu014447	Unclassified Chloroflexi		<90%
*Otu021440	Candidatus Vesicomyosocius okutanii	NR_074939.1	96%

Supplemental Figure C.3: List of the top 20 OTUs shared between sites (≥0.1%). Asterisks denotes those OTUs that are abundant (≥ 1%) in at least two sites.

VITA

Amanda Marie Achberger, a native of Baton Rouge, Louisiana, received her Bachelor of Science degree in Microbiology from Louisiana State University in May of 2010. Immediately following, she began her graduate study at Louisiana State University in the laboratory of Dr. Brent C. Christner.